

UNIVERSIDAD DE GRANADA
DEPARTAMENTO DE ECOLOGÍA



**CAPACIDAD DE RESPUESTA AL ESTRÉS
AMBIENTAL DE POBLACIONES DE *PINUS
SYLVESTRIS* Y *P. NIGRA* EN EL LÍMITE SUR
DE DISTRIBUCIÓN: UNA APROXIMACIÓN
MULTIDISCIPLINAR**

TESIS DOCTORAL

Asier Herrero Méndez

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Memoria que el Licenciado Asier Herrero Méndez presenta para aspirar al
Grado de Doctor por la Universidad de Granada

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

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Aspirante al Grado de Doctor

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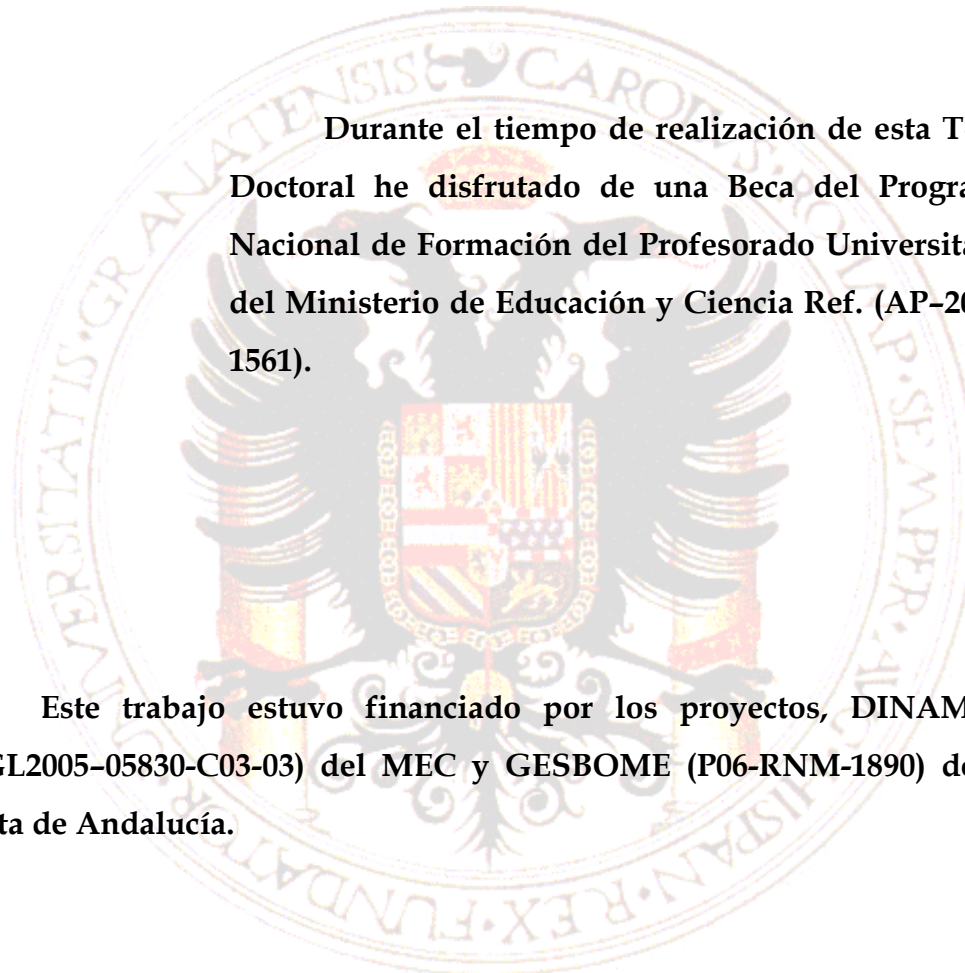
CERTIFICAN

Que los trabajos de investigación desarrollados en la Memoria de Tesis Doctoral: "Capacidad de respuesta al estrés ambiental de poblaciones de *Pinus sylvestris* y *P. nigra* en el límite sur de distribución: una aproximación multidisciplinar", son aptos para ser presentados por el Ldo. Asier Herrero Méndez ante el Tribunal que en su día se designe, para aspirar al Grado de Doctor por la Universidad de Granada.

Y para que así conste, en cumplimiento de las disposiciones vigentes, extendemos el presente certificado a 13 abril de 2012

Dr. Regino Zamora Rodríguez

Dr. Jorge Castro Gutiérrez



Durante el tiempo de realización de esta Tesis Doctoral he disfrutado de una Beca del Programa Nacional de Formación del Profesorado Universitario del Ministerio de Educación y Ciencia Ref. (AP-2005-1561).

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

A Ama, por su cariño.
A Aita, por sus enseñanzas.
A mi sorgintxo, por estar a mi lado.

Basajaunaren eremuan...

“Los ojos no ven nada más de lo que miran,
y no miran nada más de lo que ya conocen.

Añadamos como corolario
que si no encuentran lo que buscan,
dicen que no hay nada.”

Telesforo de Aranzadi (1860-1945)

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RESUMEN / ABSTRACT

RESUMEN

En la presente tesis doctoral estudiamos la respuesta ambiental de poblaciones de la especie boreo-alpina *Pinus sylvestris* y la mediterránea *P. nigra* situadas en el límite inferior de distribución bajo un escenario de cambio global. Analizamos la respuesta climática y sus posibles interacciones con la herbivoría en juveniles establecidos de ambas especies, ya que estos representan el futuro próximo del bosque y sus posibilidades de expansión. Estudiamos también la respuesta climática en adultos, con el objetivo de analizar la respuesta poblacional incluyendo los principales estados ontogenéticos. En el **capítulo 1** estudiamos la respuesta de juveniles de *P. sylvestris* y *P. nigra* a un evento de sequía extrema, analizando los cambios producidos en el crecimiento y el $\delta^{13}\text{C}$ y $\delta^{18}\text{O}$ foliar en juveniles muertos durante la sequía. La sequía extrema de 2005 provocó un evento de mortalidad masiva entre los juveniles de ambas especies, registrando *P. sylvestris* una mayor mortalidad. El mayor control estomático de *P. nigra* frente a las condiciones de sequía puede ser una de las causas detrás de su menor vulnerabilidad a la mortalidad por sequía. En el **capítulo 2** analizamos la influencia de la herbivoría en la respuesta climática de juveniles de *P. sylvestris* y *P. nigra* en el límite forestal. La herbivoría por insecto tuvo un papel poco relevante, mientras que los ungulados redujeron significativamente el crecimiento en altura de los juveniles herbivorizados, mostrando una mayor preferencia por *P. sylvestris*. Así, *P. sylvestris* se muestra como una especie más vulnerable a la sequía y a la herbivoría, que registra crecimientos similares o inferiores a *P. nigra*. En el **capítulo 3** estudiamos el impacto a largo plazo del clima y la herbivoría por ungulados en el crecimiento en altura de juveniles de *P. sylvestris* y *P. nigra* mediante un modelo jerárquico Bayesiano. El impacto positivo de la precipitación y el negativo de la herbivoría a escala individual presentan una influencia similar en zonas de bosque, no así en el límite forestal, donde la influencia de la precipitación es mayor. Los resultados indican que el efecto de la precipitación es

más homogéneo y extensivo, mientras que el de la herbivoría es más variable, concentrándose en algunos individuos muy dañados. Por otro lado, al agregar los datos individuales a escala de paisaje se sobreestima tanto el efecto de la herbivoría como el de la precipitación. En el **capítulo 4** analizamos la relación entre el clima y el crecimiento radial en adultos de *P. sylvestris* y *P. nigra* a lo largo de gradientes ambientales, contemplando también la variabilidad en el crecimiento junto a los cambios climáticos registrados en la zona de estudio. La ausencia de una influencia clara de la precipitación, exceptuando las zonas más bajas, puede deberse a factores microclimáticos, de adaptación local, o estructurales que mitiguen el incremento de la aridez detectado. Por otro lado, mientras que las temperaturas de septiembre pueden alargar la sequía estival impactando negativamente el crecimiento, temperaturas más altas en invierno y primavera favorecen el crecimiento. Finalmente, el aumento en la variabilidad del crecimiento registrado en las zonas más bajas sugiere una mayor vulnerabilidad de éstas a los futuros cambios climáticos. En el **capítulo 5** analizamos la respuesta de los pinares de montaña mediterránea frente a un evento de sequía extrema, examinando la resistencia y resiliencia del crecimiento de rama y la longitud de acícula en las especies arbóreas y arbustivas dominantes: *P. sylvestris* y *Juniperus communis* en el piso oromediterráneo, y *P. nigra* y *J. oxycedrus* en el piso supramediterráneo. Se comparan además juveniles y adultos para cada especie. A pesar de que el impacto de la sequía después de tres años fue relativamente bajo para todos los casos, se detectaron importantes diferencias entre especies y estados ontogenéticos. En general, los juveniles mostraron una mayor recuperación en el crecimiento de rama que los adultos, siendo los juveniles de *P. nigra* en el piso oromediterráneo y los de *J. oxycedrus* en el supramediterráneo, los que presentaron una mayor resiliencia en el crecimiento. Tanto juveniles como adultos de *P. nigra* mostraron una mayor resiliencia relativa en el crecimiento y la longitud foliar que *P. sylvestris* en las localidades donde ambas especies coexisten. Conjuntamente, las diferentes vulnerabilidades a la sequía y a la herbivoría detectadas para *P. sylvestris* y *P.*

nigra en el presente estudio, pueden acarrear un cambio de dominancia a favor de *P. nigra* en los pinares de montaña mediterráneos.

ABSTRACT

The present work focuses in the environmental response of boreo-alpine *Pinus sylvestris* and Mediterranean *P. nigra* rear edge populations under global change scenario. We analyze the climatic response and its possible interactions with herbivory in established saplings of the two species, as saplings represent the near-future forest and the possibilities for forest expansion. Adults' climatic response is also studied, in order to examine whole population response from the main ontogenetic states. In the **chapter 1** we study the response of *P. sylvestris* and *P. nigra* to an extreme drought event, analyzing changes in height growth and leaf $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ in saplings that die during drought. In fact, widespread sapling mortality was detected coupled to 2005 extreme drought, with *P. sylvestris* showing a higher mortality. Tighter control of stomatal conductance performed by *P. nigra* could be in part responsible for its lower vulnerability to drought-induced mortality. In the **chapter 2** we check the possible interactions between herbivory and the climatic response of *P. sylvestris* and *P. nigra* saplings in the treeline. Ungulates produced more damage than insects, showing a higher preference for *P. sylvestris* and reducing height growth in browsing saplings. Thus, *P. sylvestris* is the species most prone to suffer drought- and ungulate-induced damages, recording similar or lower growth rate than *P. nigra*. **Chapter 3** focuses in the long-term impact of climate and herbivory by ungulates in *P. sylvestris* and *P. nigra* saplings height growth, using a hierarchical Bayesian model. The positive impact of precipitation and negative of herbivory at individual level presented similar influence in the woodland, but not in the treeline, where the influence of precipitation was larger. The results indicate that the effect of precipitation is more homogeneous and pervasive at the population level, while the effect of herbivory is more variable and concentrate in some highly-damaged individuals. On the other hand, the effects of precipitation and herbivory are overestimated when the individual data are aggregated at the landscape level. In the **chapter 4** we analyze

the relationship between climate and radial growth in adults of *P. sylvestris* and *P. nigra* along environmental gradients, considering also growth variability and climatic trends in the study area. The absence of strong precipitation signals in growth, with the exception of low elevation populations, could be related to microclimatic factors, local adaptation, or stand-level structure buffering the increasing aridity conditions. While high temperatures of previous September impact negatively tree growth by lengthen summer drought, high winter and spring temperatures boost radial growth. Finally, recorded increases of growth variability in the lowest areas suggest higher vulnerability of lowlands to the future climatic changes. In the **chapter 5** we analyze the response of Mediterranean mountain pinewoods to an extreme drought event, examining resistance and resilience in shoot growth and needle length for dominant tree and shrub species: *P. sylvestris* and *Juniperus communis* at the oromediterranean belt, and *P. nigra* and *J. oxycedrus* at the supramediterranean belt. Adults and saplings were also compared for each species. Although the impact of drought after three years was relatively low for all the cases, noteworthy differences were detected between species and ontogenetic states. In general, saplings displayed better growth recovery than adults, being *P. nigra* and *J. oxycedrus* saplings, at high and low elevations respectively, the most resilient in terms of growth. Adults as well as saplings of *P. nigra* displayed higher relative resilience for growth and needle length than did those of *P. sylvestris* where both species coexist. Overall, different drought and herbivory vulnerabilities detected in this study for *P. sylvestris* and *P. nigra*, could favor a change in dominance towards *P. nigra* in the Mediterranean mountain pinewoods.

INTRODUCCIÓN GENERAL

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El término *cambio climático* engloba las variaciones del clima respecto al historial climático a escala global o regional debido a causas antropogénicas. El incremento de las temperaturas y la disminución de las precipitaciones han contribuido a sequías más largas y severas a partir de los años 70, particularmente en los trópicos y subtrópicos (Trenberth et al. 2007). Además, en el futuro se prevé un aumento en la frecuencia, intensidad y duración de las sequías extremas y olas de calor, especialmente para la región Mediterránea (Beniston et al. 2007, Christensen et al. 2007, Giorgi & Lionelo 2008).

El cambio climático es uno más de los cambios ambientales derivados de las actividades humanas, incluido en el concepto del *cambio global*. Así, el cambio global incluye cambios múltiples e interactivos entre los que destacan el incremento de CO₂ en la atmósfera y el consiguiente calentamiento global, el incremento en la fijación y deposición de nitrógeno que conlleva la alteración del ciclo del nitrógeno a escala global, y los cambios en el uso del suelo con múltiples consecuencias en los ecosistemas a diferentes escalas. Los cambios en el uso del suelo parecen tener una mayor relevancia a escala regional, influyendo de manera importante el paisaje (Terradas 2001, Valladares et al. 2004). Sus efectos sobre las interacciones bióticas pueden determinar en gran parte la respuesta de los ecosistemas a los rápidos cambios climáticos (Linares et al. 2009, Allen et al. 2010, Speed et al. 2010).

EFFECTOS DEL CAMBIO GLOBAL SOBRE LOS ECOSISTEMAS

Los motores de cambio global han afectado severamente a la estructura y funcionamiento de los ecosistemas, y en el futuro se esperan efectos aún más drásticos. Se han registrado alteraciones fenológicas (Peñuelas & Filella 2001,

Parmesan & Yohe 2003), cambios en la distribución de las especies (Parmesan & Yohe 2003, Peñuelas et al. 2007), eventos de mortalidad masivos (Allen & Breshears 1998, Allen et al. 2010), cambios en la estructura y diversidad de las comunidades (Tilman 1988, Chapin et al. 2001), y alteraciones en la disponibilidad de nutrientes en el suelo (Jonasson et al. 1999, Jensen et al. 2003).

El desplazamiento geográfico de las condiciones climáticas apropiadas para las especies vegetales ha causado migraciones hacia latitudes y altitudes más altas (Parmesan & Yohe 2003, Peñuelas et al. 2007). Así, se han registrado avances del límite forestal en diversas partes del mundo (Harsch et al. 2009). Estos cambios en la distribución pueden verse acelerados por los eventos de mortalidad masiva registrados en especies arbóreas (Allen et al. 2010). Estos eventos de mortalidad están asociados a sequías extremas y/o olas de calor, mediados en muchas ocasiones por plagas o patógenos. Posiblemente, las especies situadas en su límite meridional de distribución sean las más vulnerables a estos eventos de mortalidad, ya que se encuentran alejadas de sus óptimos ambientales.

Las interacciones bióticas pueden desempeñar también un papel importante en los cambios de distribución de las especies. La herbivoría es uno de los principales factores bióticos capaz de modelar la estructura de las comunidades, afectando al establecimiento de plántulas y el crecimiento de juveniles (Hester et al. 2006, Gill 2006), reduciendo la floración y la producción de semillas (Gómez & Zamora 2000, Mueller *et al.* 2005) e incluso alterando la disponibilidad de nutrientes (Pastor *et al.* 1998, Ritchie *et al.* 1998). Recientemente, se ha comprobado que los daños producidos por ungulados herbívoros pueden detener las expansiones altitudinales y latitudinales inducidas por el calentamiento global (Olofsson et al. 2009, Speed et al. 2010). Discernir y cuantificar el efecto de factores abióticos y bióticos sobre las comunidades y ecosistemas, puede ayudar tanto a comprender los cambios producidos, como a anticiparlos y diseñar estrategias para mitigarlos.

Muchos de los cambios registrados en la estructura y diversidad de comunidades y ecosistemas se deben a la distinta vulnerabilidad de las especies a las fuentes de estrés. En el oeste de Estados Unidos, una mayor mortalidad por sequía en las especies arbóreas que en las arbustivas está dando paso a comunidades con una mayor dominancia de estas últimas (Allen & Breshears 1998, Mueller et al. 2005, Koepke et al. 2010). De igual modo, diferencias inter-específicas en la palatabilidad pueden acarrear cambios en la composición de las comunidades debido a cambios en los tamaños poblacionales de los herbívoros, los cuales han experimentado aumentos generalizados en las últimas décadas (Husher et al. 2003, Cote et al. 2004). Además, la interacción entre factores abióticos y bióticos puede resultar determinante en la respuesta ambiental de poblaciones y comunidades (Post & Pedersen 2008). Así, el establecimiento, crecimiento y/o reproducción de una especie en particular, limitado o favorecido por las condiciones climáticas, puede verse a su vez afectado positiva o negativamente por la herbivoría, alterando la respuesta ambiental de la especie. El estudio de las vulnerabilidades especie-específicas a factores abióticos y bióticos entre especies clave con una amplia distribución, puede ser de gran ayuda a la hora de predecir futuros cambios en comunidades y ecosistemas.

LA IMPORTANCIA DEL LÍMITE SUR Y LAS POBLACIONES RELICTAS

La dinámica de las poblaciones situadas en los límites de distribución de las especies es de vital importancia a la hora de determinar y predecir los cambios en la distribución de las especies inducidos por el clima (Thomas et al. 2004, Hampe & Petit 2005). Las poblaciones del límite sur de distribución, situadas en el frente de retroceso donde se esperan importantes contracciones, son extremadamente importantes para la conservación de la diversidad genética, la historia filogenética y el potencial evolutivo de las especies (Hampe & Petit 2005). Al estar más alejadas

del las condiciones óptimas de la especie, se prevé un mayor impacto del incremento de la aridez en las poblaciones del límite inferior de distribución.

Muchas de estas poblaciones se encuentran aisladas en zonas de montaña formando poblaciones relictas, donde encuentran condiciones ambientales favorables gracias a la heterogeneidad espacial propia de zonas de montaña (Hampe & Jump 2011). Estas poblaciones relictas presentan a menudo una gran diferenciación genética y adaptación a las condiciones locales que favorecen su persistencia en zonas próximas al límite de tolerancia de las especies (Hampe & Petit 2005, Hampe & Jump 2011). A pesar de ello, numerosos estudios constatan el peligro que suponen para estas poblaciones los rápidos cambios climáticos previstos (Sarris et al. 2007, Púlido et al. 2008, Mendoza et al. 2009, Galiano et al. 2010). La Cuenca Mediterránea constituye el límite meridional para muchas especies arbóreas como *Pinus sylvestris*, *Abies alba* o *Quercus pyrenaica*, además de ser una zona especialmente sensible al cambio climático (Giorgi & Lionelo 2008).

Las poblaciones situadas en el frente de retroceso en zonas mediterráneas están fuertemente limitadas por la sequía, influenciando negativamente las principales tasas demográficas: supervivencia (Castro et al. 2004, Matías et al. 2012), crecimiento (Thabeet et al. 2009, Linares & Tíscar 2010) y reproducción (Mendoza et al. 2009, Girard et al. 2011). Sin embargo, existen diferentes procesos y factores que pueden mitigar y/o compensar la influencia negativa de la sequía en las tasas demográficas. La variación en las condiciones ambientales a pequeña escala puede proporcionar ‘puntos calientes’ de regeneración (Quero et al. 2008) y de supervivencia (Lloret et al. 2004). La tolerancia al estrés ambiental, la plasticidad fenotípica y las interacciones de facilitación pueden también mitigar el efecto negativo de las sequías extremas y el paulatino incremento de la aridez (Lloret et al. 2012). Por otro lado, las nuevas condiciones climáticas pueden resultar favorables para las especies afectadas. El aumento de las temperaturas de primavera puede adelantar el inicio de la época de crecimiento compensando en

parte las condiciones desfavorables al final de la época estival (Richter & Eckstein 1990, Richter et al. 2012). Así, el futuro de las poblaciones situadas en el límite inferior latitudinal dependerá del balance entre el impacto negativo de los rápidos cambios climáticos y el efecto positivo de los mecanismos de estabilización y compensación demográfica.

LA IMPORTANCIA DE LA FASE ONTOGENÉTICA

La respuesta de las especies vegetales a los cambios climáticos previstos puede variar en gran medida según el estado ontogenético considerado. La respuesta ambiental a nivel individual varía con la edad, debido al incremento de tamaño y al aumento de la complejidad estructural (Thomas & Winner 2002, He et al. 2005). Los cambios asociados al incremento de tamaño incluyen el aumento del volumen de suelo explorado por el sistema radicular, que permite el acceso a fuentes de agua y nutrientes más profundas (Clark 1983). Por otro lado, el incremento en altura aumenta la distancia a la que deben de ser transportados hormonas y asimilados fotosintéticos (Maggs 1964). Estos cambios pueden conllevar una distinta sensibilidad entre juveniles y adultos frente a eventos climáticos extremos como las sequías extremas (Donovan & Ehleringer 1994, Cavender-Bares & Bazzaz 2000). La respuesta global de la población a los rápidos cambios climáticos previstos estará determinada por la integración de la respuesta en cada una de sus fases. Por ejemplo, una baja resistencia a las sequías extremas en la fase de plántula puede ser irrelevante para la dinámica poblacional, si la resistencia es alta en adultos y juveniles. El estudio de la respuesta poblacional a los cambios climáticos comparando sus principales estadios ontogenéticos, puede ser clave a la hora de predecir la futura composición de la comunidad.

En contraste con los numerosos estudios realizados con plántulas y juveniles de corta edad en ambientes Mediterráneos (Gómez-Aparicio et al. 2004,

Castro et al. 2004, Quero et al. 2011, Matías et al. 2012), los juveniles pre-reproductores ya establecidos son una fase poco estudiada en las especies Mediterráneas. Estos suponen una fase crítica para el estudio de la respuesta poblacional a las condiciones ambientales, ya que representan el futuro inmediato del bosque. El papel de los juveniles establecidos es clave en el actual contexto de cambio global, donde las sequías reiteradas pueden provocar eventos de mortalidad masivos (Allen et al. 2010). Así, la resistencia de los juveniles a las sequías extremas y su posterior crecimiento, puede favorecer la persistencia de poblaciones y comunidades. De igual modo, el comportamiento de los juveniles es fundamental en las esperadas migraciones altitudinales y latitudinales como consecuencia del desplazamiento de las condiciones climáticas. Por lo tanto, los juveniles establecidos suponen una pieza clave en la capacidad de regeneración y expansión del bosque.

ÁREA DE ESTUDIO Y ESPECIES

La zona de estudio de la presente tesis doctoral está ubicada en el Parque Natural Sierra de Baza (Granada; 2°51' O, 37°22' N), exceptuando el capítulo 1, que se llevo a cabo en el Parque Nacional de Sierra Nevada (Granada; 3°28' O, 37°5'' N). El clima Mediterráneo de la Sierra de Baza se caracteriza por inviernos fríos y veranos cálidos, con una prolongada sequía estival (Junio-Agosto). La nieve es frecuente durante los meses de invierno, pudiendo persistir incluso más de dos meses, especialmente a partir de los 1800 m de altitud. La Figura 1 muestra el diagrama climático para el periodo 1920-2007, elaborado con la base de datos de alta resolución CRU TS 2.1 (Mitchell & Jones 2005). La precipitación total media es de 482 ± 12 mm y la temperatura media de 12.2 ± 0.1 °C.

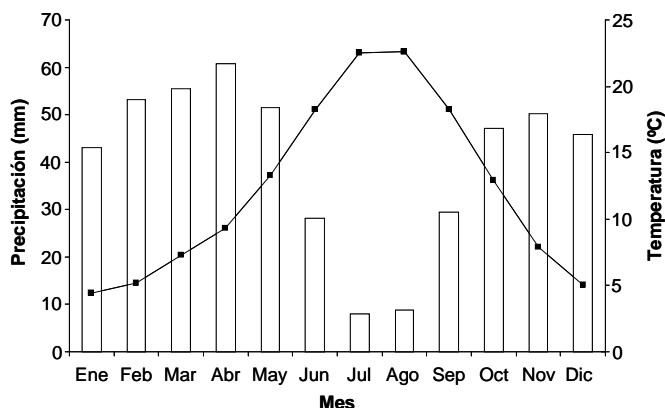


Figura 1 Diagrama climático de la Sierra de Baza para el periodo 1920-2007.

La zona de estudio está situada en la parte norte del Parque Natural, donde se encuentran los bosques naturales relictos de *Pinus sylvestris* L. y *Pinus nigra* Arnold (Figura 2). El sustrato de la zona está constituido principalmente por calizas. *P. sylvestris* y *P. nigra* presentan una amplia distribución natural (Figura 3 y 4), y han sido frecuentemente utilizadas en reforestaciones y plantaciones tanto en la cuenca Mediterránea como en otras partes de Europa, ocupando sólo en España 1.700.000 ha (MARM 2006). Mientras que *P. sylvestris* es una especie de origen biogeográfico boreo-alpino, *P. nigra* es una especie marcadamente Mediterránea. Ambas especies crecen en la cuenca Mediterránea en áreas montañosas a altitudes medias y altas, pudiendo llegar a formar grandes extensiones (Bárbero et al. 1998). Es común encontrar poblaciones contiguas de ambas especies, ocupando *P. sylvestris* ubicaciones más elevadas y/o exposiciones más frías y húmedas (Blanco et al. 1997). *P. sylvestris* encuentra en la Sierra de Baza, junto a Sierra Nevada, su límite meridional absoluto (Boratynski 1991), dónde soporta duras condiciones ambientales (Castro et al. 2004). Para *P. nigra*, la población de la Sierra Baza es una de las más meridionales de la Península Ibérica.

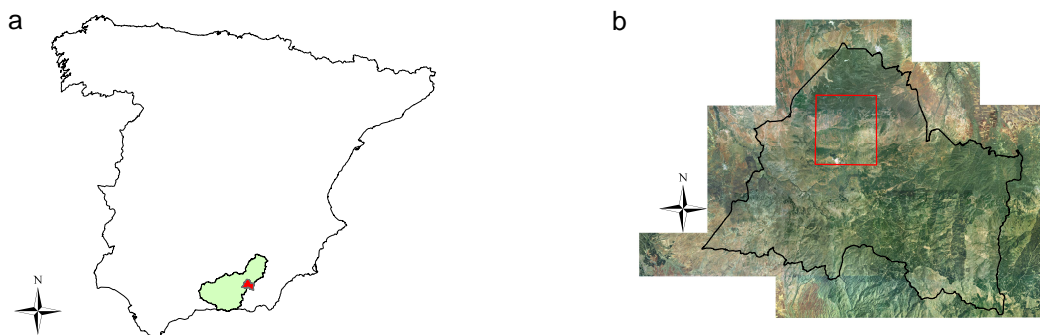


Figura 2 (a) Localización del Parque Natural Sierra de Baza (en rojo), situado en la provincia de Granada (en verde). (b) Ortofoto del Parque Natural, dónde se ubica la zona de estudio (delimitada en rojo).

La Sierra de Baza alberga además un gran número de ungulados silvestres y domésticos. El ciervo (*Cervus elaphus* L.) fue reintroducido en el Parque Natural en 1988, mostrando su población un gran incremento debido a la ausencia de depredadores y a una inapropiada gestión cinegética (Granados et al. 2001). Hoy en día presenta una población aproximada de 2600 individuos (5,04 ind/km²; CMA 2008). En cambio la cabra montés (*Capra pyrenaica* Schinz) mantiene tan sólo una pequeña población en el parque (0,39 ind/km²; CMA 2009). El ganado doméstico, compuesto por cabras (*Capra aegagrus hircus* L.) y ovejas (*Ovis aries* L.), aumenta considerablemente la presión herbívora durante la temporada de pastoreo (Mayo-Diciembre). La densidad del ganado doméstico en la zona de estudio se ha mantenido constante en la última década (40 ind/km²; CMA 2008). Estudios previos en el área de estudio han demostrado el impacto negativo de los ungulados en el crecimiento y reclutamiento de especies como *P. sylvestris* (Zamora et al. 2001, Gómez et al. 2001).

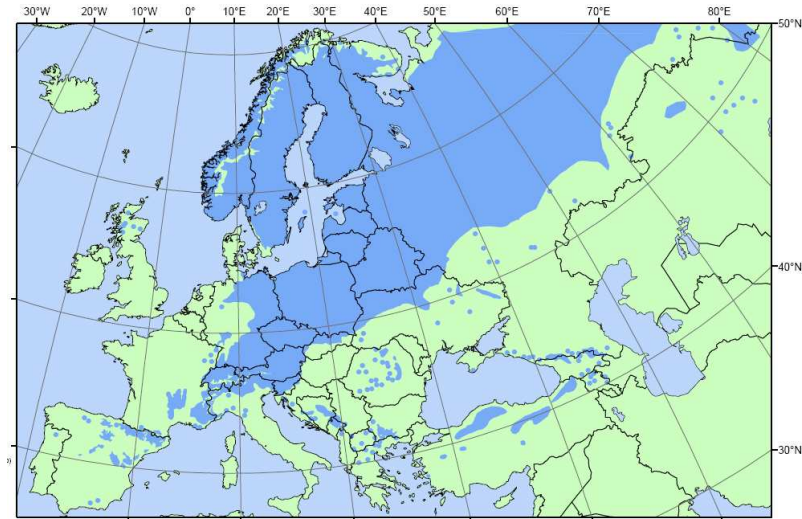


Figura 3 Distribución natural de *P. sylvestris*. Mapa obtenido de www.euforgen.org

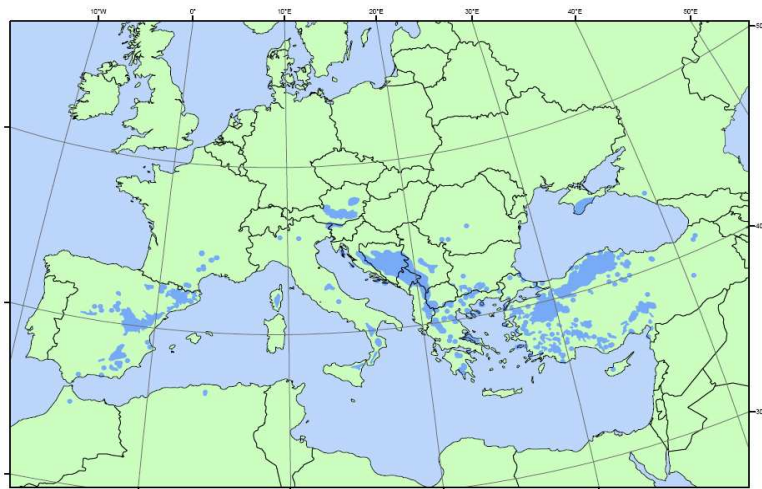


Figura 4 Distribución natural de *P. nigra*. Mapa obtenido de www.euforgen.org

OBJETIVO DE LA TESIS Y ESTRUCTURA EN CAPÍTULOS

El objetivo de la presente tesis doctoral es analizar la respuesta de dos especies clave con contrastado origen biogeográfico como *P. sylvestris* y *P. nigra* a

fuentes de estrés tanto abiótico como biótico en el límite sur de distribución. En el presente trabajo analizamos la respuesta de las dos especies a factores climáticos como la temperatura y la precipitación, además de a la sequía extrema de 2005 acaecida en las zonas de estudio. Por otro lado, estudiamos el impacto de la herbivoría por ungulado, así como su peso frente a las variables abióticas. El análisis del impacto de la sequía y la herbivoría en variables como el crecimiento y la supervivencia, nos permitirá evaluar la respuesta de poblaciones relictas situadas en el frente de retroceso. ¿Podemos esperar una respuesta aclimatada de estas poblaciones debido a factores de compensación propios de poblaciones relictas? ¿O por el contrario presentarán una alta vulnerabilidad debido a la cercanía a su límite de tolerancia?

En cuanto a la diferente vulnerabilidad al estrés entre las dos especies, esperamos una peor respuesta a la sequía extrema por parte de *P. sylvestris*, debido a su origen biogeográfico boreal. De igual manera, prevemos una mayor incidencia de daños por herbivoría en *P. sylvestris* debido a su mayor palatabilidad (Baraza et al. 2009). La respuesta diferencial de estas dos especies al estrés ambiental, puede conllevar cambios en la composición de los pinares Mediterráneos formados por ambas especies, con un paulatino desplazamiento de *P. sylvestris* por parte de *P. nigra* en áreas hasta hoy dominadas por la especie boreal.

Los diferentes aspectos de este estudio se analizan en 5 capítulos diferentes, cada uno de ellos con un objetivo específico:

Capítulo 1. Objetivo: estudiar la respuesta de juveniles de *P. sylvestris* y *P. nigra* a un evento de sequía extrema. Para ello se analizan los cambios producidos en la composición isotópica del carbono ($\delta^{13}\text{C}$) y oxígeno ($\delta^{18}\text{O}$) foliar en juveniles muertos durante la sequía y en aquellos que sobrevivieron, estimando así parámetros clave en la respuesta al estrés hídrico como la eficiencia en el uso del agua y el control estomático. También se analizan las diferencias en las tasas de mortalidad por sequía en las dos especies. Estos datos nos permitirán comprobar las

diferencias en la vulnerabilidad a la sequía por parte de las dos especies y analizar el comportamiento fisiológico de ambas frente a condiciones de sequía extrema.

Capítulo 2. Objetivo: estudiar la influencia de la herbivoría en la respuesta climática de juveniles de *P. sylvestris* y *P. nigra* en el límite forestal. Para ello cuantificamos los daños causados por herbívoros insectos y ungulados, así como su preferencia por las dos especies estudiadas. También se comparan las tasas de crecimiento de ambas especies, como un indicador de la respuesta a las condiciones abióticas. Todo ello con un diseño espacial que aísla en gran medida las características de cada especie del entorno inmediato, seleccionando parejas de individuos de edad y tamaño similar. Los resultados nos permitirán aproximarnos al papel de la herbivoría en el límite forestal, una zona de potencial expansión del bosque, muy dinámica en el actual escenario de cambio climático.

Capítulo 3. Objetivo: estudiar el impacto a largo plazo del clima y la herbivoría en el crecimiento de juveniles de *P. sylvestris* y *P. nigra*. Para ello usamos un modelo jerárquico Bayesiano, analizando el efecto del clima y la herbivoría en el límite forestal y en zonas de bosque durante 16 años. Comparamos los resultados a escala individual y a escala de paisaje, cuantificando la aportación de la variabilidad climática y de la herbivoría en la respuesta de los juveniles en ambos entornos. Los resultados posibilitarán analizar la pérdida de información que ocurre al agregar los datos provenientes de la escala individual, a la cual ocurren los procesos de respuesta ambiental, en la escala de paisaje, de la que usualmente se infieren los patrones de respuesta.

Capítulo 4. Objetivo: analizar la relación entre el clima y el crecimiento radial en adultos de *P. sylvestris* y *P. nigra* a lo largo de gradientes ambientales. Analizamos la relación clima-crecimiento mediante correlaciones y funciones respuesta, analizando también sus variaciones temporales junto con los cambios climáticos

registrados en la zona de estudio. El análisis de la relación clima-crecimiento en el límite de distribución asociado a la sequía posibilitará la identificación de las variables climáticas que más limitan el crecimiento, y nos ayudará a predecir el impacto de los cambios climáticos previstos en estas poblaciones. La comparación entre distintas poblaciones posibilitará la identificación de las zonas más vulnerables al progresivo incremento de la aridez.

Capítulo 5. Objetivo: analizar la respuesta de los pinares de montaña Mediterránea de *P. sylvestris* y *P. nigra* frente a un evento de sequía extrema. Se examina la resistencia y resiliencia del crecimiento de rama y la longitud de acícula en las especies arbóreas y arbustivas dominantes en la zona: *P. sylvestris* y *Juniperus communis* L. en el piso oromediterráneo, y *P. nigra* y *J. oxycedrus* Sibth & Sm en el piso supramediterráneo. Además, se comparan juveniles establecidos con individuos adultos para cada una de las especies. Los resultados nos permitirán explorar las diferencias en resistencia y resiliencia entre especies y estadios ontogenéticos, siendo de gran valor a la hora de predecir la dinámica de la comunidad en un escenario con alta frecuencia de sequías extremas.

Los resultados de la presente tesis doctoral posibilitarán una mejor comprensión de los impactos de los rápidos cambios climáticos en las especies, así como su interacción con factores bióticos de gran relevancia como la herbivoría. También serán de gran ayuda a la hora de diseñar estrategias para mitigar el impacto negativo de estos cambios en la montaña Mediterránea, donde muchas especies encuentran su límite de distribución más meridional, y donde se prevén grandes pérdidas de diversidad.

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CHAPTER 1:

DIFFERENTIAL RESPONSE OF SAPLINGS OF TWO

COEXISTING PINE SPECIES TO AN EXTREME DROUGHT

EVENT

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Delgado-Huertas



ABSTRACT

Drought-induced events of massive tree mortality appear to be increasing worldwide under climate change scenario. Species-specific vulnerability to drought mortality in mature adults may alter patterns of species diversity, while little studied tree sapling mortality could also constraint forest regeneration. We explore the consequences of 2005 extreme drought causing high sapling mortality (approx. 50 %) among 10-year-old saplings of two coexisting pine species in the Mediterranean mountain: boreo-alpine *P. sylvestris* and Mediterranean *P. nigra*. Height growth and leaf $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and nitrogen were measured in the last four leaf cohorts in saplings that died during drought and in those that survived. Relations between measured variables indicate that photosynthetic activity was mainly limited by stomatal conductance (g_s) in the two species. Dead saplings displayed a great increase of leaf $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ respect to previous years, with extreme high $\delta^{13}\text{C}$ values suggesting an important role of g_s reduction in mortality. While *P. nigra* decreased its growth in response to drought conditions, *P. sylvestris* maintained similar growth level. Thus, lower growth coupled to higher increase of leaf $\delta^{18}\text{O}$ in dead *P. nigra* saplings indicate a more conservative water use, in contrast to lower sensitivity of *P. sylvestris*. This different physiological behavior could influence 2005 mortality rates, which contributed to higher cumulative drought mortality for *P. sylvestris* since the time of planting. The observed species-specific drought vulnerability could lead to fast changes in dominance, diversity, and distribution in Mediterranean mountain pinewoods under the predictions of increased frequency of extreme drought events.

Keywords: drought stress, heat stress, tree mortality, leaf $\delta^{13}\text{C}$, leaf $\delta^{18}\text{O}$, Mediterranean mountain forest

INTRODUCTION

Extreme droughts are recurrent in many climate types worldwide (FIC 2006; Seager et al. 2007; Trenberth et al. 2007). Episodes of severe drought can have an impact on woody plant communities in several ways, weakening plant performance and even provoking shrub or tree mortality (Peñuelas et al. 2001; Breshears et al. 2005; Bigler et al. 2006; Miriti et al. 2007). This, in turn, may alter patterns of species diversity and dominance (*sensu* Walker et al. 1999), with strong consequences for forest structure and functioning (Allen and Breshears 1998; Mueller et al. 2005; Slik 2004).

Massive tree mortality is one of the most drastic and apparent consequences of extreme droughts (Allen et al. 2010). Episodes of widespread tree mortality are likely to increase in large areas of the planet under the current scenario of climatic change, with elevated temperatures and/or water stress as a common implicated causal factor (Fensham and Holman 1999; Bréda et al. 2006; Bigler et al. 2006; van Mantgem and Stephenson 2007; Bentouati 2008). Massive tree mortality may trigger far-reaching changes in ecosystem function and structure, alter carbon dynamics (Jones et al. 2009) and ecosystem water budgets (Huxman et al. 2005), change near-ground solar radiation (Martens et al. 2000), reduce biotic associations (Mueller et al. 2005), and diminish tree-regeneration capacity (Allen and Breshears 1998; Slik 2004). Furthermore, different drought vulnerability among species may alter patterns of dominance and even accelerate climate-driven distributional shifts, with strong implications for forest diversity and composition (Allen and Breshears 1998; Slik 2004; Breshears et al. 2005).

Studies on the effect of extreme droughts on tree mortality have focused largely on adult individuals (Allen et al. 2010 and references within). However, studies on the effect of extreme drought events on sapling mortality are scant (but see Suarez and Kitzberger 2008; Floyd et al. 2009; Lingenfelder and Newbery 2009). While adult tree mortality can result in current community changes (Slik

2004; Breshears et al. 2005) sapling mortality can block forest regeneration and expansion to new areas. Indeed, tree recruitment depends largely on windows of opportunity with particular favorable conditions that happen with low frequency (Jelinski and Cheliak 1992). An increase in the frequency of climatic-induced widespread sapling mortality events may thus cancel recruitment pulses of tree species. This may be particularly critical for Mediterranean-type and semi-arid ecosystems, given the increase in frequency and severity of extreme droughts that is occurring (and predicted) at regional scales (Moreno 2005; FIC 2006; Briffa et al. 2009), coupled with the low frequency of windows of opportunity for effective tree recruitment in these ecosystems (Holmgren and Scheffer 2001; García and Zamora 2003; Mendoza et al. 2009).

In this study, we analyze the way in which saplings of two coexisting pine species with contrasting biogeographical origins, *Pinus sylvestris* L. and *Pinus nigra* Arnold, respond to an unusual and extreme drought event. *Pinus sylvestris* is a boreo-alpine species widely distributed in Eurasia and restricted to high mountains areas in the Mediterranean basin (Boratynski 1991), while *P. nigra* is a widespread Mediterranean species (Barbéro et al. 1998). Today *P. sylvestris* and *P. nigra* occupy large areas in the northern Mediterranean basin, both growing in mountainous areas at medium to high elevations (Blanco et al. 1997, Barbéro et al. 1998). It is common for populations of the two species to be contiguous, usually with *P. sylvestris* at higher elevations or in colder, fresher exposures (Barbéro et al. 1998; Blanco et al. 1997). A differential ability to cope with extreme droughts might thus alter the composition, dominance, and distribution of these Mediterranean pine forests.

In 1997, we conducted an experiment to analyze survival and growth of *P. sylvestris* and *P. nigra* in Sierra Nevada National Park, SE Spain (Castro et al. 2002). Pines were two years old at the time of planting. In 2005, the most extreme drought in the last 46 years occurred in the area, exacerbated by a particularly hot, dry summer. This extreme drought can thus be considered a natural experiment that

affected a field experiment with pines 10 years old at the time of the drought. This offered the opportunity to check the impact of extreme droughts in two coexisting species experimentally controlling age and ecological conditions. For this purpose, we monitored ecological and ecophysiological variables linked to drought stress in saplings of both species: survival, performance and leaf carbon and oxygen isotopic composition ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$).

Stable isotopic signatures provide time-integrated information on plant responses to changing abiotic conditions (Dawson et al. 2002), offering a valuable tool to analyze plant responses to extreme drought events. $\delta^{13}\text{C}$ constitutes an integrated record of the ratio of intercellular to atmospheric CO_2 concentrations (c_i/c_a) during the period in which the carbon was fixed (Farquhar et al. 1982, Pate 2001). Thus, $\delta^{13}\text{C}$ is a good indicator of intrinsic water-use efficiency (WUE_i), reflecting the balance between net photosynthetic rate (A) and stomatal conductance (g_s). On the other hand, plant $\delta^{18}\text{O}$ is influenced by the isotopic composition of source water, evaporation and diffusion processes occurring in leaves, and isotopic exchange between oxygen atoms in organic molecules and plant water (Barbour et al. 2007). As $\delta^{18}\text{O}$ is also inversely related to the ratio of atmospheric to intercellular water vapour pressure (e_a/e_i), it is strongly affected by g_s , and have been widely used as time-integrated measure of g_s when variation in source water $\delta^{18}\text{O}$ is minimum (Querejeta et al. 2008, Ramirez et al. 2009, Hasselquist et al. 2010, Moreno-Gutierrez et al. 2012). Further, the combined measurement of plant $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ allows discrimination between biochemical and stomatal limitations to photosynthesis (Scheidegger et al. 2000, Grams et al. 2007, Sullivan and Welker 2007, Querejeta et al. 2008), because $\delta^{18}\text{O}$ is related to g_s but is unaffected by A , while $\delta^{13}\text{C}$ is affected by both.

Long leaf life-span and unique annual flush of needle growth in targeted species allows measurements of inter-annual variability in WUE_i inferred by stable isotope signatures, and therefore comparison between leaf cohorts formed during contrasted conditions (i.e. extreme drought vs ‘normal conditions’). We also

sampled individuals that died during drought and those that survived in order to disentangle the causes of mortality. Although much progress was made on physiological mechanism involved in tree death following drought in the last years (McDowell et al. 2008, Adams et al. 2009, Breshears et al. 2009), the specific mechanisms leading to tree to mortality are far to being resolved (Sala et al. 2010, McDowell and Sevanto 2010). McDowell et al. (2012) suggest that hydraulic function and carbohydrate and defense metabolism are highly interdependent, rather than mutually inclusive but independent mechanisms. Studies analyzing responses of trees to drought after mortality or during dead process such the present work are scant (but see Breshears et al. 2009, Adams et al. 2009), and could help to clarify mechanisms underlying climate-driven vegetation mortality.

Finally, the objectives of the study are: 1) To find any difference in explored variables between dead and live saplings which could help in future research of drought-induced mortality 2) To determine the response of each species to an extreme drought event. We hypothesize that the boreo-alpine species will be less able to cope with extreme drought than the Mediterranean species, showing a higher mortality rate, lower growth, and higher drought stress inferred by leaf $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$.

MATERIAL AND METHODS

STUDY AREA

The study was carried out at the locality of Loma Panaderos (N 37° 04' 50'' W 3° 27' 50''), situated at 1825 m. a.s.l. in the Sierra Nevada National Park (SE Spain). The climate is continental Mediterranean, characterized by a severe summer drought. The annual precipitation is 860 ± 86 mm (mean for 1997-2006 period), but the summer mean (months of June, July and August) is only 35 ± 9 (mean for

1997-2006 period; data from a climatic station placed at La Cortijuela Botanical Garden, at 800 m from the study area). The year 2005 was the driest of the last 46 years (Mendoza *et al.* 2009), and the summer of 2005 was especially hot and dry (Fig. 1). A drought index (DRI) was calculated for the study site to summarize differences in moisture conditions among years that cover the entire lifespan of pine saplings (1997-2006). The DRI was calculated using the following formula:

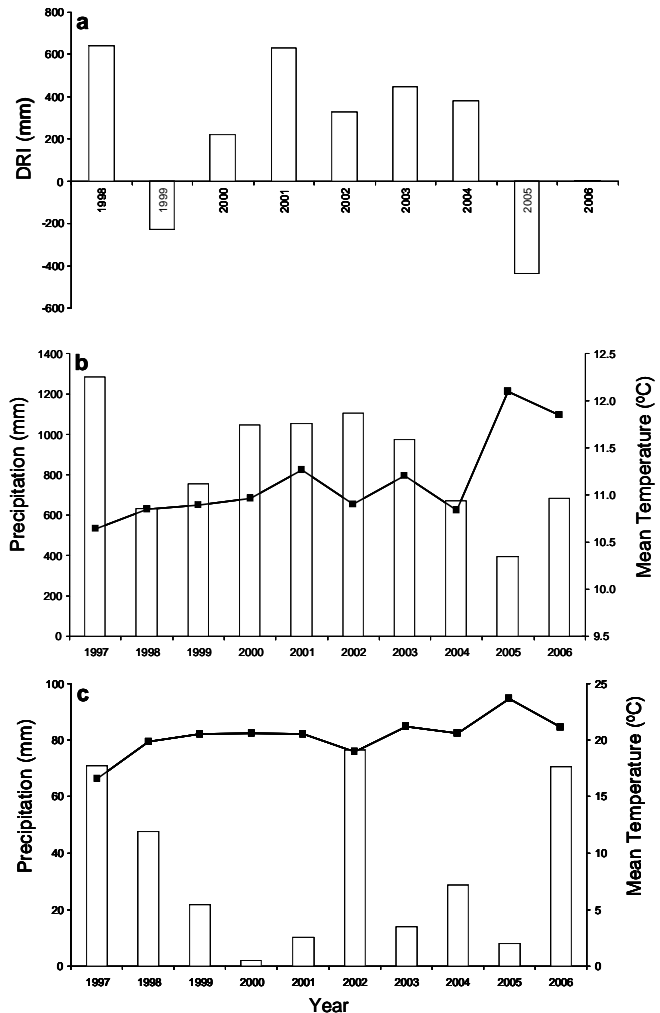
$$\text{DRI} = \text{P} - \text{PET}$$

where P is equal to the sum of the precipitation of August (previous year) to July (current year), and PET equals the sum of estimated potential evapo-transpiration for the same period as a function of monthly mean temperatures and geographical latitude (Thornthwaite 1948). We chose the same period as Bigler *et al.* (2006), given the similarities in the seasonally dry conditions and phenology of shoot growth for both study areas. A value of DRI below zero indicates moisture deficit. DRI data are shown in Figure 1.

The study area was burned by an accidental fire in 1983, and was reforested in 1997 with two-year-old seedlings of *P. sylvestris* and *P. nigra* that were planted within three fenced areas of ca. 0.9 ha (see Castro *et al.* 2002 for details). The vegetation before the fire was a mixed forest of the two pine species. Saplings were permanently tagged and monitored for survival since the time of planting (Castro *et al.* 2004, present study). The cumulative sapling mortality due to drought in 2001 was 75.2 % for *P. sylvestris* and 53.3 % for *P. nigra* ($\chi^2 = 36.42$, $P < 0.0001$; modified from Castro *et al.* 2004). From 2001 to 2004 there was almost no mortality (less than % 2 for the two species). However, coupled with the extreme drought of 2005, high mortality of saplings of both species was recorded in late summer (August-September). Dead saplings showed no signals of insect or pathogen damage, or mechanical injury, neither during the study year or the previous years. In the study area there was not tree mortality events related with bark beetle outbreaks, in opposite to other European areas (Dobbertin *et al.* 2007). Pine processionary caterpillar (*Thaumetopoea pityocampa* Den. et Schiff.) could

cause tree mortality after three consecutive years of defoliation, but tree mortality related to its outbreak in the area is rather low (less than 6 % during 5 years, Hóðar *et al.* 2003).

Figure 1 Drought index (a), annual (b) and summer (c) total precipitation and mean temperature for 1997-2006 series, the time period covering the entire lifespan of pine saplings. Drought index encompasses precipitation and PET values from previous August to current July, thus just covering the period 1998-2006. See text for a complete definition of the index.



SAPLING MORTALITY AND PERFORMANCE

In the first week of 2006 March, dead and living saplings of both species were counted in the fenced areas to quantify the mortality rate. From each species, 20 live and 20 dead individuals were recorded at random for one of the fenced areas.

Sapling height, basal diameter, and internode growth during the last four years (2005, 2004, 2003, and 2002) were measured. Relative height growth (RHG) values were calculated for each measured sapling using the following formula (Leopold and Kriedemann 1975):

$$RHG = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \text{ (year}^{-1}\text{)}$$

where W_2 and W_1 are sapling heights at times 2 and 1, respectively.

LEAF $\delta^{13}\text{C}$ AND $\delta^{18}\text{O}$ ANALYSIS

A random subsample of 12 saplings per species and condition (dead/alive) from the 20 measured were chosen to perform the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ analysis. Needles from the last four years (2005, 2004, 2003, and 2002) were collected at the same time from the axis stem in the second week of 2006 March. The needles were stored in paper envelopes and oven dried at 60°C for 48 h. Then, needles were ground and analyzed for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$. Leaf $\delta^{13}\text{C}$ analyses were performed at the Biogeochemistry of Stable Isotopes Laboratory of the Experimental Centre of Zaidín (Granada, Spain), using a mass spectrometer (Thermo Finnigan Delta plus XP). Leaf $\delta^{18}\text{O}$ analyses were conducted at the Stable Isotope Facility of the University of California-Davis using a Heckatech HT Oxygen Analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK), following the method described in Kornexl et al. (1999). Leaf samples were converted by pyrolysis in a glassy carbon reactor at 1400°C to CO and H₂O, and oxygen was analyzed as CO.

The abundance of stable isotopes is presented in delta notation (δ), relative to a standard:

$$\delta = \left(\frac{R_{\text{samp}}}{R_{\text{st}}} - 1 \right) \times 1000 \text{‰}$$

where R is the molar ratio of the heavy to light isotopes ($R = {}^{13}\text{C}/{}^{12}\text{C}$). R_{samp} refers to the sample, and R_{st} to the international standard Vienna-Pee Dee Belemnite for $\delta^{13}\text{C}$ and Vienna Standard Mean Oceanic Water for $\delta^{18}\text{O}$. The working standards for $\delta^{13}\text{C}$ were cellulose and phthalic acid, and for $\delta^{18}\text{O}$ microcrystalline cellulose. The repeated analysis of these internal standards yielded a standard deviation of less than 0.1‰. Analyses of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ were performed on whole-leaf tissue rather than extracted cellulose, because of strong positive correlations observed between $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of whole tissue and cellulose (West et al. 2001; Sullivan and Welker 2007, Powers et al. 2008). Since all the individuals were located in the same area and belong to two species of the same genus, we assume that the atmospheric CO_2 concentration was similar for all of them and that the variation in leaf $\delta^{13}\text{C}$ due to differences in leaf-to-air vapor-pressure deficit between the two species was low.

LEAF N ANALYSIS

Leaf nitrogen concentration (N) is considered an indicator of maximum carboxylation capacity (Field and Mooney 1986) and is positively related to the photosynthetic capacity (Reich et al. 1995), as the greatest part of foliar N is linked to photosynthetic machinery (Evans and Seemann 1989). Therefore, we analyzed leaf N to test for differences in carboxylation capacity among species and leaf cohorts. The assay was performed for the samples used to analyze $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ (see above), using an elemental analyzer Leco CNH TrueSpec (Michigan, USA).

DATA ANALYSIS

Differences in mortality rate between both species were analyzed using a nominal logistic regression. Height, basal diameter and RHG were compared between living and dead individuals as saplings' growth was finished prior to mortality event (authors' personal observation). Differences in height and basal diameter were examined using a one-way ANOVA, and RHG using a multivariate ANOVA with dependent variable as a repeated measure. Year (leaf cohort) was the repeated factor, and species, condition (live/dead), species crossed with condition, and all interactions with year were sources of variation.

Leaf $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and N data were analyzed separately for live and dead saplings, as they represent different phenological states. While dead saplings stopped suddenly their metabolism due to mortality in late summer, live saplings' isotopic signatures could be influenced by environmental conditions after drought. Nevertheless, as our sampling took place in winter shortly after summer drought, we expect few differences due to sampling lag, especially for $\delta^{13}\text{C}$, which mainly reflect c_i/c_a during the leaf formation (Farquhar et al. 1982, Pate 2001). In addition, low winter temperatures of the study site may limit photosynthetic activity, reducing possible enrichment of both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ after summer drought. Leaf $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and N data were submitted to a multivariate ANOVA with year as the repeated factor, and species, and its interaction with year as sources of variation.

Post-hoc comparisons between species for different years in RHG, leaf $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and N were performed using t-tests and applying a Bonferroni correction to significance level (32 comparisons, $\alpha = 0.0016$). In addition, Pearson's correlation coefficient was used to analyze paired relationships between RHG, leaf $\delta^{13}\text{C}$, and $\delta^{18}\text{O}$, considering each pine sapling and each year separately for the two species and conditions. Significant correlation's slopes were compared between species and conditions using standardized major axis test. All the analyses were performed using JMP 7.0 (SAS Institute Inc.), except comparisons of correlations'

slopes, which were performed using SMATR software (Falster et al. 2006). All results throughout this paper are given as mean \pm standard error.

RESULTS

MORTALITY AND PERFORMANCE

After the 2005 extreme drought, the cumulative drought mortality was significantly higher ($\chi^2 = 23.98$, $P < 0.001$) for *P. sylvestris* (89.8 %, $n = 378$) than for *P. nigra* (76.0 %, $n = 402$). Despite 2005 mortality rate was also higher for *P. sylvestris* (62.74%, $n = 83$) than for *P. nigra* (48.26%, $n = 169$), no significant differences were found ($\chi^2 = 2.48$, $P = 0.1155$).

Mean height of the saplings at the end of the 2005 growing season was 79.75 ± 4.05 cm for *P. sylvestris* and 74.36 ± 2.68 cm for *P. nigra*, and the mean basal trunk diameter was 2.69 ± 0.14 cm and 2.86 ± 0.14 cm, respectively. There were no significant differences in height and basal diameter between living and dead saplings within the species ($P > 0.05$ for all the cases). Also, no significant differences were found between dead and live saplings for RHG ($F = 0.06$, $P = 0.8106$). However, species displayed significant differences in RHG ($F = 5.38$, $P = 0.0231$), although these differences varied with considered year (significant year * species interaction, $F = 12.23$, $P < 0.0001$; see Fig. 2). In extremely drought 2005, *P. nigra* lowered its height growth respect to 2004, while *P. sylvestris* maintain a similar growth level. In fact, RHG of 2005 was significantly larger for *P. sylvestris* ($p < 0.0016$, Fig. 2).

LEAF $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ AND N

Leaf $\delta^{13}\text{C}$ varied significantly between years for both live and dead individuals of the two species, displaying an increase in 2005 respect to 2004, which was stronger for dead saplings (Table 1, Fig. 2). Between live saplings this increase was more apparent in *P. nigra*, resulting in significantly higher leaf $\delta^{13}\text{C}$ than *P. sylvestris* in 2005 (Table 1, Fig. 2). Leaf $\delta^{18}\text{O}$ also varied significantly between years, with dead saplings of the two species displaying a large increase in 2005 respect to the previous years (Table 1, Fig. 2). *P. nigra* dead saplings showed significantly higher leaf $\delta^{18}\text{O}$ than dead *P. sylvestris*, especially in 2005 (Table 1, Fig. 2). Leaf N also varied significantly depending of the considered year, showing a decreasing trend with age (Fig. 2). No significant differences between the two species were detected in leaf N across years (Table 1, Fig. 2).

Table 1 Summary of the multivariate ANOVA results for leaf carbon and oxygen isotopic composition ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$, ‰), and leaf nitrogen content (N, ‰) for live and dead saplings.

Source	Live			Dead		
	df	F	P	df	F	P
Leaf $\delta^{13}\text{C}$						
<i>Between subjects</i>						
Species	1	5.57	0.0275	1	0.07	0.7996
<i>Within subjects</i>						
Time (Year)	3	87.38	<0.0001	3	410.06	<0.0001
Time x Species	3	3.77	0.0271	3	2.61	0.0801
Leaf $\delta^{18}\text{O}$						
<i>Between subjects</i>						
Species	1	0.06	0.8173	1	17.45	0.0006
<i>Within subjects</i>						
Time (Year)	3	8.52	0.0015	3	69.48	<0.0001
Time x Species	3	1.70	0.2099	3	2.97	0.0629
Leaf N						
<i>Between subjects</i>						
Species	1	0.23	0.6355	1	1.61	0.2182
<i>Within subjects</i>						
Time (Year)	3	29.56	<0.0001	3	42.31	<0.0001
Time x Species	3	1.99	0.1476	3	0.63	0.6089

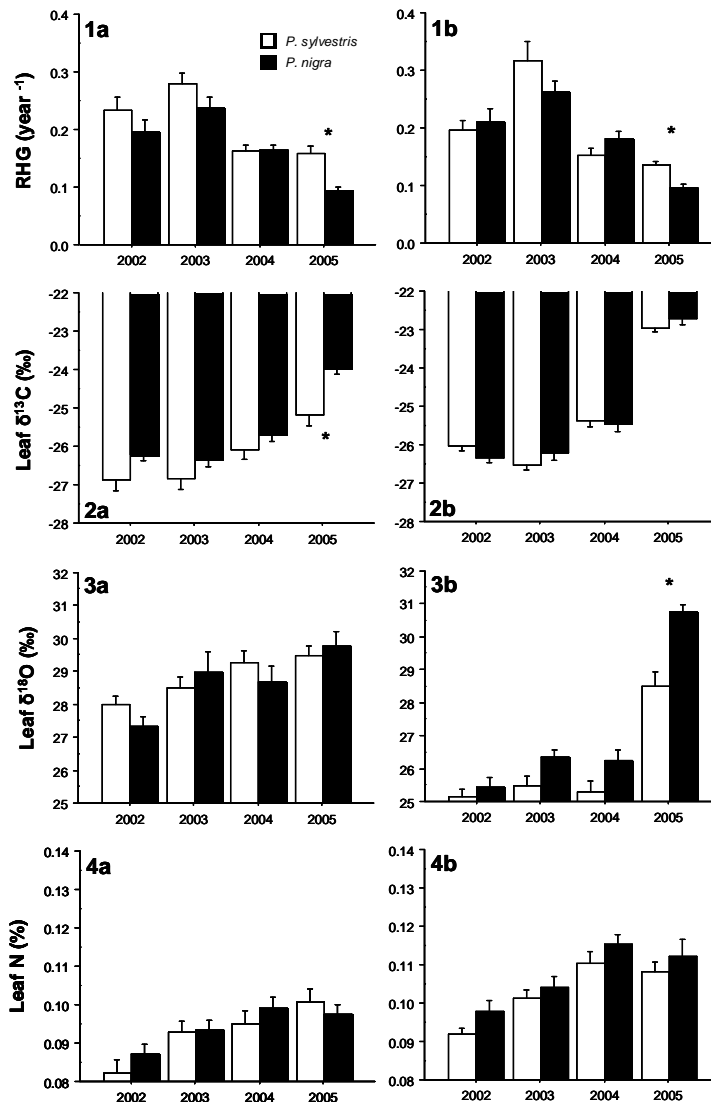


Figure 2 RHG (1), leaf $\delta^{13}\text{C}$ (2), leaf $\delta^{18}\text{O}$ (3) and leaf N (4) for live (a) and dead (b) saplings of *P. sylvestris* and *P. nigra* in the last four years (2002-2005). (*) indicate significant differences between groups ($P < 0.0016$). Bars indicate the standard errors of calculated means. $N = 20$ for RHG, and $N=12$ for the rest of variables

RELATIONSHIPS BETWEEN LEAF $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ AND RHG

The leaf $\delta^{13}\text{C}$ was positively correlated with $\delta^{18}\text{O}$ for the two species and the two conditions, being significant for dead saplings and marginally significant for live saplings (Fig. 3). No significant differences were found between slopes of

the two species for dead saplings (SMATR test statistic = 0.54, $P = 0.468$). In addition, significant negative correlations were recorded between leaf $\delta^{13}\text{C}$ and RHG for the two species and conditions (Fig. 4). Although correlation coefficients were higher for *P. nigra* than for *P. sylvestris*, and for dead than for live saplings, no significant differences in slopes were recorded between species and conditions ($P > 0.05$ for all the cases). Finally, significant negative correlations between leaf $\delta^{18}\text{O}$ and RHG were found for dead saplings of both species (Fig. 5), with no significant differences in slope between species (SMATR test statistic = 0.81, $P = 0.399$).

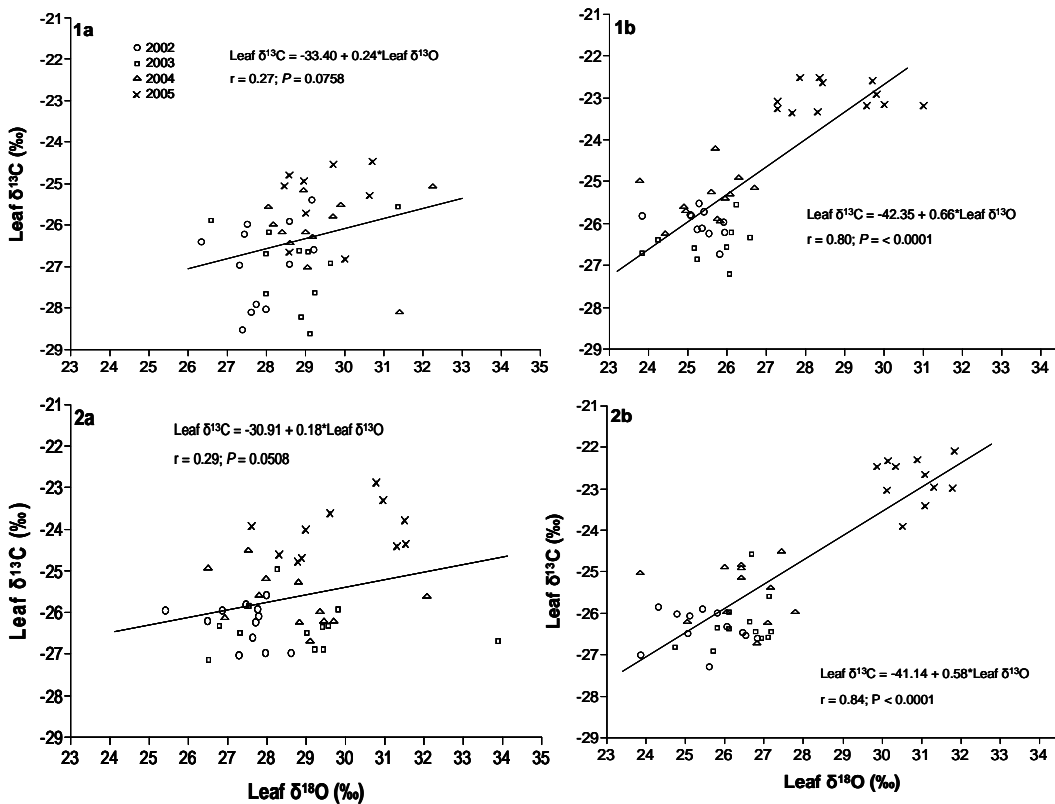


Figure 3 Pearson's correlations between leaf $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ for live (a) and dead (b) saplings of *P. sylvestris* (1) and *P. nigra* (2) considering each pine sapling. $N = 48$

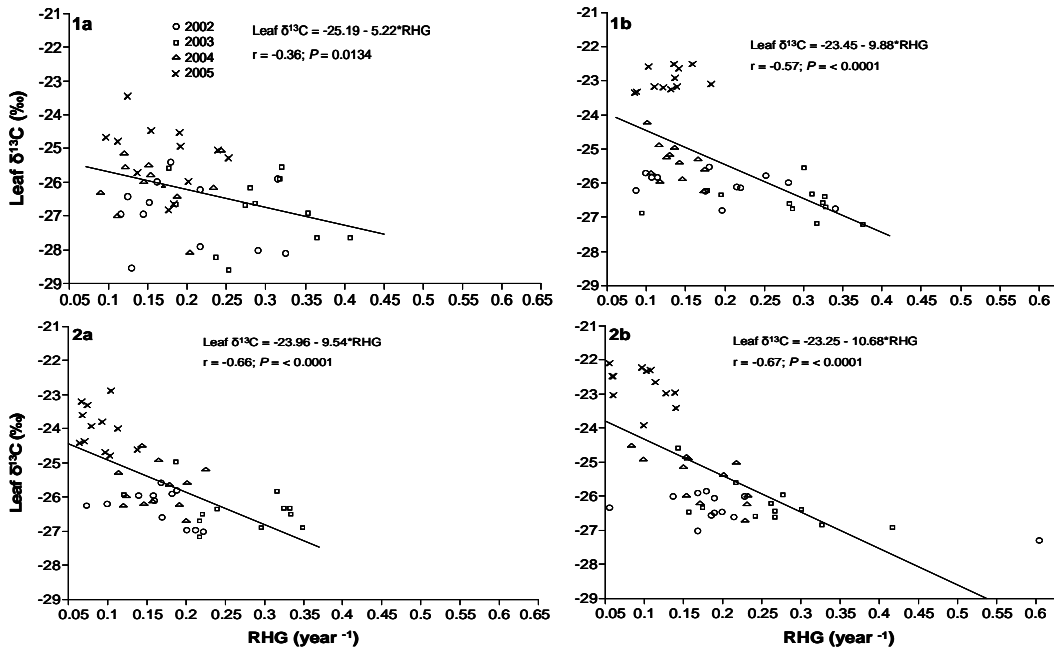


Figure 4 Pearson's correlations between leaf $\delta^{13}\text{C}$ and RHG for live (a) and dead (b) saplings of *P. sylvestris* (1) and *P. nigra* (2) considering each pine sapling. $N = 48$

DISCUSSION

2005 extreme drought cause a widespread mortality in saplings, which represents near-future forest composition. Recorded mortality rates of 2005 contributed to differences in cumulative drought mortality between the two species, with *P. sylvestris* showing higher mortality. Stomatal limitation was the main constraint to photosynthetic activity for the two species, as indicated by positive correlations between leaf $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ (Scheidegger et al. 2000, Grams et al. 2007) and negative ones between leaf $\delta^{13}\text{C}$ and RHG. Thus, the general increase in leaf $\delta^{13}\text{C}$ in response to 2005 drought conditions was mainly driven by reductions in g_s . Despite common patterns found in dead and live saplings in the main role of stomatal limitation and increase of leaf $\delta^{13}\text{C}$ in response to drought, they represent different phenological conditions. While dead saplings' isotopic signature reflect

time-integrated gas-exchange until 2005 late summer, labile constituents of leaf tissue in live saplings could be affected by autumn and winter conditions. Therefore, we discuss *P. sylvestris* and *P. nigra* response to 2005 drought separately for live and dead saplings.

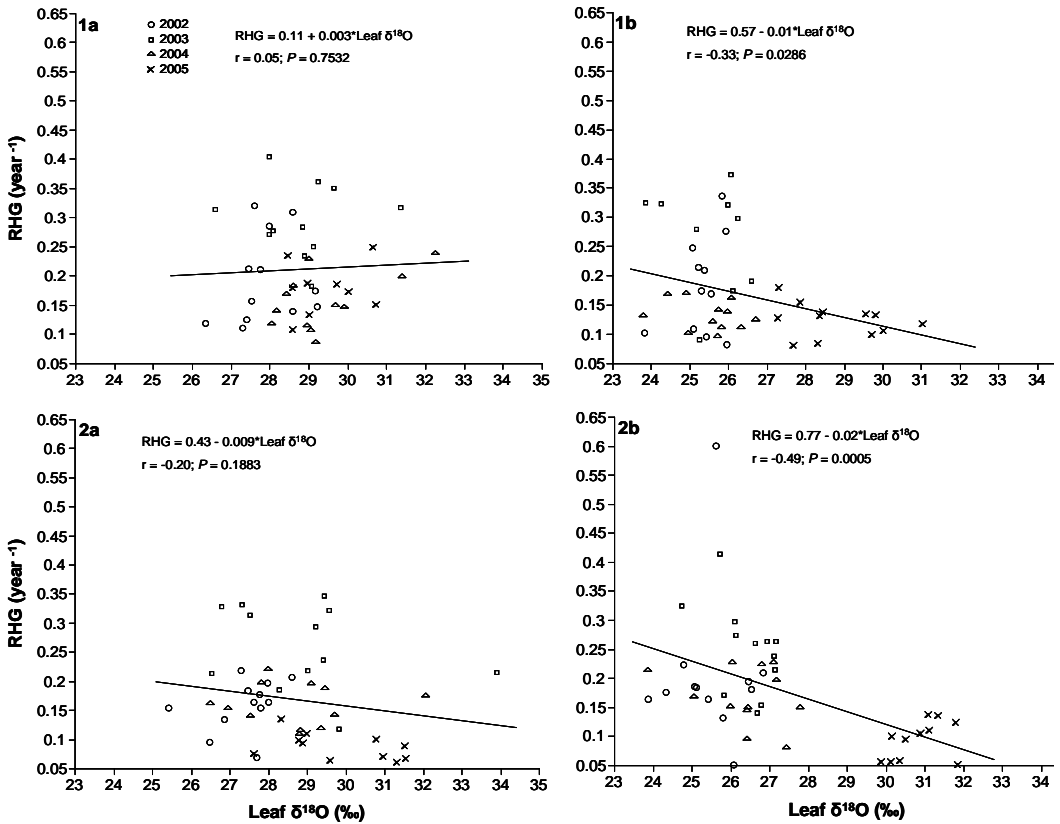


Figure 5 Pearson's correlations between RHG and leaf δ¹⁸O for live (a) and dead (b) saplings of *P. sylvestris* (1) and *P. nigra* (2) considering each pine sapling. N = 48

DROUGHT RESPONSE IN DEAD SAPLINGS

Leaf δ¹³C and δ¹⁸O values of dead saplings offer valuable information as they reflect isotopic signatures at the time of death. Recorded increases of leaf δ¹⁸O

in 2005 for dead saplings indicate a strong reduction of g_s in response to drought conditions, entailing extremely high 2005 leaf $\delta^{13}\text{C}$ values, achieving maximum values close to -22 ‰. These values were much higher than those recorded for *P. sylvestris* seedlings under experimental drought conditions (Cregg and Zhang 2001). In addition, recorded values were generally similar than those recorded for other pine species from more arid zones (Lajtha and Getz 1993, Van de Water et al. 2002, Oliveras et al. 2003, Querejeta et al. 2008), also under extreme drought conditions which trigger widespread drought-induced mortality events (Adams and Kolb 2004, Koepke et al. 2010). Therefore, leaf $\delta^{13}\text{C}$ values for *P. sylvestris* and *P. nigra* during 2005 drought were extremely high and could be linked to observed mortality, considering that similar $\delta^{13}\text{C}$ values were commonly recorded in drier semi-arid environments and under especially dry conditions.

Apparent increases of drought-induced forest mortality events throughout different biomes (Allen et al. 2010) have triggered the interest in mechanisms of tree mortality during drought. For species showing isohydric behavior, such *P. sylvestris* and *P. nigra* (Irvine et al. 1998, Cinnirella et al. 2002), plant carbon balance seems to play an important role due to tight stomatal control during drought (McDowell et al. 2008). This agrees with observed role of g_s in studied saplings. The absence of any pest or pathogen in the present study prevent confounding effects in drought-induced mortality by biotic agents, as insect outbreaks could cause tree mortality independent of drought effects (McDowell et al. 2008). The consequences of 2005 drought could be exacerbated by high temperatures of that summer, as heat stress can upset carbon balance as a result of the higher cost of tissue maintenance (Amthor 2000; Hartley et al. 2006) and accelerated cellular repair process (Atkin and Macharel 2009). Higher carbon demand, coupled with low carbon assimilation because of diminished g_s and drought impact on phloem transport (Sala et al. 2010), may translate as cellular-level carbon starvation leading saplings to dead. Nevertheless, catastrophic hydraulic failure cannot be ruled out as the cause of mortality. In fact, carbon

metabolism and hydraulic function seems to be highly interdependent processes (McDowell et al. 2012), which can be linked with g_s and WUE_i .

Although dead saplings of the two species responded to drought conditions of 2005 with a strong decrease of g_s , *P. sylvestris* and *P. nigra* displayed different responses that could affect observed differences in mortality rate. Firstly, *P. nigra* recorded a decrease of RHG in 2005 respect to previous year, while *P. sylvestris* maintain a similar growth level. Secondly, *P. nigra* showed in 2005 higher leaf $\delta^{18}O$ than *P. sylvestris*, indicating a higher g_s reduction. In contrast, saplings of the two species showed similar leaf $\delta^{13}C$. Following the dual isotope conceptual model (Scheidegger et al. 2001, Grams et al. 2007), similar $\delta^{13}C$ but higher $\delta^{18}O$ for *P. nigra* indicate higher reduction in both g_s and A. However, the absence of differences in leaf N between the two species suggested little differences in maximum carboxylation capacity (Field and Mooney 1986). In a greenhouse study about the influence of light intensity in the photosynthetic rate, both species displayed a similar photosynthetic response, with low differences in A (Fernández and Tapias-Martín 2004). Thus, it seems that *P. nigra* performed a conservative water use through tight control of g_s in response to water shortage conditions, thereby reducing its height growth. In contrast, *P. sylvestris* performed a more prodigal use of water with less control over g_s , maintaining previous year growth level in 2005 drought.

Observed species-specific physiological behavior in response to drought stress could influence recorded mortality rates and affect establishment success of the two species. *P. nigra* was more sensitive to drought than *P. sylvestris*, modulating its WUE_i in response to moisture conditions. While higher WUE_i is considered an advantageous characteristic in dry areas due to a conservative water use (Akhter et al. 2005, Yin et al. 2005, Yang et al. 2010), lower WUE_i coupled to similar photosynthetic rate implies less control over g_s , increasing vulnerability to cavitation events and hydraulic failure mortality (McDowell et al. 2008). However, a trade-off might exist for high WUE_i values inferred by leaf $\delta^{13}C$ under extreme

drought conditions. Although high WUE_i through tight stomatal control avoid hydraulic failure with a more wide safety margin, extremely high values could diminish carbon assimilation pushing saplings close to cellular-level carbon starvation. Recorded mortality rates in 2005 extreme drought for the two species contributed to observed differences in cumulative drought mortality since the time of planting, being survival 2.4 times higher for *P. nigra* than for *P. sylvestris*. In summary, the boreo-alpine species *P. sylvestris* is less resistant to drought than the Mediterranean *P. nigra*, and these differences persist for the sapling bank under extreme dry conditions.

DROUGHT RESPONSE IN LIVE SAPLINGS

Although in a lesser degree, an increase of leaf $\delta^{13}C$ in 2005 drought was also recorded for live saplings, particularly for *P. nigra*. However, no concomitant increases in leaf $\delta^{18}O$ were recorded in 2005. It is possible that live saplings undergo additional drought stress after sapling mortality event, due to dry autumn and winter (drier than the mean for 1997-2006 period, data not shown) after 2005 summer. This could produce an overall leaf $\delta^{18}O$ enrichment, masking the increase in $\delta^{18}O$ due to drought. Finally, lower height growth and higher increase of leaf $\delta^{13}C$ for *P. nigra* in 2005 respect to previous year, agrees with described more conservative water use for *P. nigra* than for *P. sylvestris*.

CONSEQUENCES FOR FOREST DYNAMICS

Massive mortality of adult trees related to climate-induced physiological stress seems to be increasing under the current scenario of global warming (Allen et al. 2010). In this context, our results support the idea that processes of massive mortality may also occur at the sapling stage, even if adults survive the extreme drought; adult tree mortality did not increase in the study area (authors' personal

observation) despite the high sapling mortality. Sapling sensitivity to drought events might be higher than that of adults due to a shallower root system, while lower carbon reserves increase the risk of death from water deficit (Hanson and Weltzin 2000). This may be particularly true for open areas where the forest has the potential for expansion, but lacks an adult tree canopy that could buffer climatic extremes.

The different response of both species to 2005 extreme drought and massive sapling mortality observed in this study could have important consequences for the forest dynamics and distribution of these pines. Mortality events in tree saplings could delay and/or block regeneration in seasonally dry forests that may already be threatened by difficulties in seedling establishment (Mendoza et al. 2009). Thus, forest expansion to early successional habitats (as represented by our study area) and even altitudinal migration due to warming (Jump and Peñuelas 2005; Lenoir et al. 2008) could be hampered by sapling mortality events due to recurrent extreme droughts (IPCC 2007). In fact, *P. sylvestris* has its southernmost distribution in mountains of the Mediterranean Basin, far from its optimal environmental conditions, and its recruitment in these Mediterranean mountains is already seriously constrained by summer drought (Castro et al. 2005; Mendoza et al. 2009). In contrast, the results show that *P. nigra* appears to have greater survival and establishment under a global-warming context. In addition, *P. nigra* has higher seed dispersal and colonization capacity of open habitats in comparison with *P. sylvestris* (Debain et al. 2007; Boulant et al. 2008). All this could lead to a fast change in regeneration capacity in pinewoods towards *P. nigra* in areas where the two species coexist, altering therefore patterns of species diversity, dominance and distribution in Mediterranean forest ecosystems.

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CHAPTER 2:
LIMITS OF PINE FOREST DISTRIBUTION AT THE
TREELINE: HERBIVORY MATTERS

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ABSTRACT

Herbivores can affect future forest composition by feeding selectivity. At temperature-sensitive treelines, herbivory can exacerbate or constrain climate-driven distributional shifts in tree species. The present study analyses the impact of herbivory in a Mediterranean treeline of widespread *Pinus sylvestris* and *P. nigra* pinewoods, testing whether herbivory damage reinforces or inhibits the climatic responses of these trees. We used naturally occurring sapling pairs of similar size and age of both species, thereby isolating plant characteristics from environmental effects in herbivore behaviour. Herbivory damage by ungulates proved higher than that caused by insects in saplings of both species. Low plant density and extreme abiotic conditions at the treeline could in part be responsible for the observed low incidence of insect herbivory. Ungulates preferred *P. sylvestris* over *P. nigra*, implying heavier browsing damage for a large number of *P. sylvestris* saplings, suffering reduced internode growth as a consequence. In addition, *P. sylvestris* could not compensate height-growth reductions due to browsing with higher growth rate than *P. nigra*. In fact, *P. sylvestris* showed similar or lower relative height growth with respect to *P. nigra*. Under a scenario of increasing aridity and maintenance of ungulate populations, the upward migration of *P. sylvestris* in its southern range could be restricted by higher drought vulnerability than *P. nigra*, a situation exacerbated by ungulate herbivory. Our results indicate that ungulate herbivory reinforces climatic response of coexisting *P. sylvestris* and *P. nigra* at treeline, favouring a potential change in community dominance towards Mediterranean *P. nigra*.

Keywords: ungulate browsing, insect herbivory, treeline dynamics, Mediterranean mountain, *Pinus sylvestris*, *Pinus nigra*

INTRODUCTION

Herbivores can affect future forest composition by feeding selectivity and/or differential response to damage by plant species (Schowalter *et al.* 1986; Danell *et al.* 2003; Cote *et al.* 2004). In fact, several studies report a reduction of recruits of the more palatable or sensitive species, and/or increase of unpalatable or tolerant species (Tilghman 1989; Vasconcelos and Cherrett 1997; Ritchie *et al.* 1998; Kay and Bartos 2000; Rooney and Waller 2003; Potvin *et al.* 2003). Therefore, if herbivores exert a differential impact on dominant plant species of a forest ecosystem, they could determine vegetation dynamics (Davidson 1993), blocking forest expansion (Speed *et al.* 2010) or changing tree-species composition (Danell *et al.* 2003).

Herbivory and its influence in plant-community dynamics could be a noteworthy factor in ecotones and the margins of species ranges, where rapid distributional changes have been detected in recent years in response to increasing warmer conditions (Parmesan and Yohe 2003; Peñuelas *et al.* 2003). Climatic conditions which limit or promote establishment of a particular tree species at the edge of its distribution could be exacerbated or inhibited by herbivory, directly influencing its performance and/or recruitment, or altering the competitive ability of a coexisting species. For example, a tree species at its southernmost distribution limit could present limited establishment due to drought impairing height growth, this in turn being exacerbated by herbivory damage diminishing photosynthetic tissue. On the other hand, another species in its uppermost distribution limit could find favourable conditions for its establishment mediated by higher rainfall, but inhibited by repeated severe browsing or defoliation. Therefore, the analysis of the impact of herbivory at the limits of species ranges could help in the assessment of range shifts under the current climatic-change scenario (Speed *et al.* 2010; Olofsson *et al.* 2009).

Treelines are temperature-sensitive ecotones between forest and alpine vegetation states (Korner and Paulsen 2004), where tree-species distributional changes have already been recorded and further are expected in response to increasing warmer conditions (Harsch *et al.* 2009). Studies demonstrating the effect of herbivory exacerbating or constraining such distributional changes have been conducted at high latitudes (Cairns and Moen 2004; Dalen and Hofgaard 2005; Tømmervik *et al.* 2009; Speed *et al.* 2010), but little attention has been paid to water-stressed areas such as Mediterranean ecosystems, where marked changes in plant-community composition are expected in response to drought and warming (Thuiller *et al.* 2005). In fact, many tree species have their southern distribution limit in the Mediterranean Basin, and thus might be more vulnerable to increasing aridity (IPCC 2007) as they are far from optimum conditions (Hampe and Petit 2005).

The aim of the present work is to analyse the impact of herbivory at the Mediterranean treeline of widespread mixed pinewoods of *Pinus sylvestris* L. and *P. nigra* Arnold (Blanco *et al.* 1997; Barbéro *et al.* 1998), testing whether herbivory damages reinforce or restrain the climatic response of these species. While *P. sylvestris* is a boreo-alpine species reaching its southern distribution limit in the Mediterranean basin (Boratynski 1991), *P. nigra* is a Mediterranean pine species distributed mainly in southern Europe (Barbéro *et al.* 1998). The study focuses on saplings, as this ontogenetic state represents the near-future forest and the possibilities for forest expansion and treeline advance. First of all, we compare sapling performance between the two species to explore species responses to climatic conditions. Then, we analyse herbivory impact and the associated guild of herbivores in saplings of both species. Since the study area is at the treeline, we expect a higher impact of vertebrate herbivores on pine saplings, due to temperature limitation for insects (Bale *et al.* 2002), and larger body size, individual bite size and mobility of vertebrates (Danell and Bergström 2002). Regarding the species studied, previous work in the area showed *P. sylvestris* to be

a more palatable species for ungulates than *P. nigra* (Baraza *et al.* 2009). Therefore, we predict greater damages at least for this herbivore guild in *P. sylvestris*. Within this framework, the specific objectives of this study are: 1) to test the relative impact of vertebrates and invertebrates on pine saplings; 2) to test the preference by herbivores for the two target species.

MATERIAL AND METHODS

STUDY SITE AND SAMPLING DESIGN

The study was conducted at Sierra de Baza Natural Park (SE Spain, 2°51'48''W, 37°22'57''N). Sierra de Baza, together with Sierra Nevada, represents the absolute southernmost distribution limit for *Pinus sylvestris* (Boratynski 1991). The climate is Mediterranean, characterized by cold winters and hot summers, with pronounced summer drought (June-August). Precipitation is concentrated mainly in autumn and spring. The annual and summer rainfall is 495 ± 33 mm and 31 ± 9 , respectively (mean \pm SE for 1991-2006 period; Cortijo Narvez meteorological station, 1360 m a.s.l.). The bedrock is predominantly calcareous. Both wild and domestic ungulates are present in Sierra de Baza. Among wild ungulates, red deer (*Cervus elaphus* L.) population reaches a density of 15.35 individuals km⁻² (CMA 2008), whereas Spanish ibex (*Capra pyrenaica* Schinz) maintain only a small population of 0.39 individuals km⁻² (CMA 2009). On the other hand, from May to December, herds of domestic goat (*Capra hircus* L.) and sheep (*Ovis aries* L.) notably intensify the grazing pressure with a density of 40 individuals km⁻² (CMA 2008).

The study was performed in three different sites of approximately 1 ha, at least 900 m apart from each other in three different valleys. All three sites are situated at ca. 2000 m of elevation with a south-west orientation. These sites are forest ecotone areas, close to mixed forests of both species, representing the

treeline for both *P. sylvestris* and *P. nigra* in the Mediterranean mountain. Therefore, selected sites represent areas for potential forest expansion towards higher altitudes. The woody vegetation of the study sites is formed mainly by saplings (defined as non-reproductive individuals) of *P. sylvestris* and *P. nigra* of different ages and sizes, with some scattered individuals of *Juniperus communis* L., *J. sabina* Sibth & Sm and *Erinacea anthyllis* Link (see Table 1 for further details about sites). In October 2008, we choose 30 pairs of both species at each site (a total of 180 pines). Each pair of saplings contained one individual from each species of similar age and size, being the closest sapling in the surrounding area (see Table 1 for further details about selected pairs). Therefore, each pair represents a set of one individual of each species growing under similar conditions, in order to test herbivore preference for *P. sylvestris* and *P. nigra*.

Table 1 Location and characteristics of the selected sites and pairs of saplings. Geographical position, sapling density, and relative proportion of *P. sylvestris* and *P. nigra* are related to whole sites. Longitudinal distance and height and age differences between saplings of selected pairs are related to pairs of saplings of each site. Values are shown as mean \pm standard error

Variable	Site		
	1	2	3
Geographical position	2°51'42.841''W 37°22'23.16''N	2°50'33.874''W 37°22'27.091''N	2°50'48.359''W 37°21'59.927''N
Sapling density (ind ha ⁻¹) ^a	318 \pm 61.3	174 \pm 47.9	250 \pm 42
Relative proportion (<i>P. sylvestris</i> / <i>P. nigra</i> ; %) ^a	72.3 \pm 0.8 / 27.7 \pm 0.8	62.4 \pm 2.1 / 37.6 \pm 2.1	54.2 \pm 10.3 / 45.8 \pm 10.3
Distance (m) ^b	1.19 \pm 0.18	2.05 \pm 0.29	1.26 \pm 0.24
Age difference (years) ^c	1.7 \pm 0.18	1.7 \pm 0.26	1.83 \pm 0.25
Height difference (cm) ^c	43.23 \pm 7.21	60.37 \pm 8.99	43.23 \pm 6.13

^a Sapling density and relative proportion of *P. sylvestris* and *P. nigra* were measured with 10 transects of 50 m length and 10 m wide

^b Longitudinal distance between saplings of selected pairs

^c Height and age differences between saplings of selected pairs. Those values are calculated using absolute values, as differences were not always in the same direction

PLANT PERFORMANCE

Height and axis internode growth of the last 11 years (2008-1998) were measured in each selected pair of saplings. Both species showed one flush per year in the study area, so we were able to estimate height corresponding to 11 years earlier based on measurements of annual internode growth. Annual internode growth (or trunk elongation) is easily identifiable by yearly whorls and bud scars. Relative Height Growth (RHG) for 2008-1998 series was calculated for each sapling using the following formula (Leopold and Kriedemann 1975) as a measure of long-term sapling performance:

$$RHG = \frac{\ln W_2 - \ln W_1}{t_2 - t_1}$$

where W_2 and W_1 are sapling heights at times 2 and 1, respectively. Age was estimated counting the number of annual bud scars or whorls (Edenius *et al.* 1995; Zamora *et al.* 2001; Debain *et al.* 2007). The estimated age ranged from 14 to 31 years and height from 0.27 to 4.05 m. The estimated age and height of saplings of each pair is very similar (see Table 1 for mean differences in age and height for each site). Age and height differences between individuals of each pair were evenly distributed among the three sites.

UNGULATE HERBIVORY

Browsing damage was recorded for the selected paired saplings in October 2008, after summer, the main browsing period in the study area (Zamora *et al.* 2001). The intensity of ungulate herbivory was quantified using the following estimates: 1) risk of herbivory, 2) damage intensity, and 3) leader browsing. Risk of herbivory

was estimated as the percentage of herbivorized plants in 2008 (annual) and in the last three-four years (accumulated) for the total of sampled individuals. Damage intensity was estimated as the proportion of apical shoots consumed by ungulates. This was quantified by counting the number of apical shoots as well as the number of apical shoots herbivorized by ungulates (shoots browsed in 2008 for annual damage intensity, and shoots browsed in the last three-four years for accumulated damage intensity). Leader browsing was estimated as the percentage of terminal leader shoots lost to herbivory. This type of herbivory caused a loss in the apical dominance of the pine sapling. We estimated the leader browsing of the last 11 years (1998-2008 series). Old losses of apical dominance were detected by clear bifurcation or strong changes of direction in the main stem (therefore a conservative measurement).

INSECT HERBIVORY

All selected paired saplings were checked for current insect damage. For damages caused by *Retinia resiniella* L. (*Lepidoptera: Tortricidae*) the number of galls were counted per sapling. We considered galls formed in 2008 and 2007 due to their two-year generation cycle (Kletecka 1992). In the case of *Rhyacionia duplana* Den. et Schiff. (*Lepidoptera: Tortricidae*), we counted the number of shoots affected by the feeding of the caterpillar in each sapling. Affected shoots were hook shaped from severe deformation (Dajoz 2001). For *Thaumetopoea pityocampa* Den. et Schiff. (*Lepidoptera: Notodontidae*), we counted the number of silk nests, which contain the larvae aggregations (Dajoz 2001). Finally, we measured the damage caused by leaf feeders belonging to *Acantholida* (*Hymenoptera: Pamphiliidae*) and *Diprion* (*Hymenoptera: Diprionidae*), estimating the percentage of needles affected of the total for the entire individual.

DATA ANALYSIS

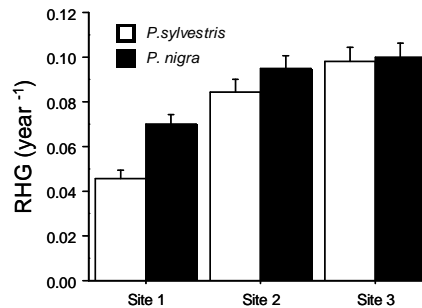
Differences in herbivory risk were analysed using a Generalized Linear Model (GLM) with a Poisson probability distribution and logarithmic link function. RHG, damage intensity and leader browsing were analysed with Generalized Linear Mixed Models (GLMM), with species and site as fixed factors and pair as a random factor. For the RHG, we pooled browsed and unbrowsed saplings, as no differences between them were recorded. While log-transformed RHG was analysed with a Gaussian probability distribution and identity function, damage intensity and leader browsing were analysed with a binomial probability distribution and a logit link function. The significance of individual fixed effects was determined by sequentially removing fixed effects from the model and comparing AIC values. In addition, the likelihood ratio test (analysis of deviance) was applied to test models against one another in ascending order of complexity. Finally, annual internode growth of the last four years (2005-2008), standardized by internode age, was compared for browsed and unbrowsed saplings of each species using a MANOVA, with the dependent variable as a repeated measure. Year was the repeated factor, and category (browsed/unbrowsed) and category interaction with year sources of variation. Saplings of the three sites were pooled for this analysis. Internode growth was transformed using the natural logarithm (\ln) to improve homogeneity of variances. All the analyses except GLMM were performed using JMP 7.0 (SAS Institute Inc.). GLMM were performed with R software (R Development Core Team 2010, version 2.11.1), using lme4 package (Pinheiro and Bates 2000). All results through out this paper are given as mean \pm standard error.

RESULTS

PLANT PERFORMANCE

Significant differences in RHG for 2008-1998 series were found between the different sites (Table 2; $t = -2.95$ and $t = -3.25$, $P < 0.05$). In addition, the differences between species varied with the site considered as shown by the significant site * species interaction (Table 2; $t = -3.26$ and $t = -4.41$, $P < 0.05$). *P. nigra* showed higher values than *P. sylvestris* in site 2 and especially in site 1, while similar values were detected in site 3 (Fig. 1). No differences between browsed and unbrowsed saplings (pooled for this analysis) were found, perhaps because we measured damage intensity only for the last three-four years while the calculated RHG encompassed the last 11 years.

Figure 1 Relative Height Growth (RHG; year⁻¹) for 1998-2008 series for *Pinus sylvestris* and *P. nigra* saplings. Untransformed data are shown to allow easy interpretation. Bars indicate the standard errors of calculated means (n = 30)



UNGULATE HERBIVORY

P. sylvestris presented a significantly higher risk of herbivory than *P. nigra* (Fig. 2), for both accumulated ($\chi^2 = 18.66$, $P < 0.0001$) and annual herbivory ($\chi^2 = 32.58$, $P < 0.0001$). In addition, significant differences were recorded between the sites for accumulated ($\chi^2 = 9.47$, $P = 0.0088$) and annual herbivory ($\chi^2 = 7.11$, $P = 0.0286$; Fig. 2).

Table 2: Model selection for accumulated and annual damage intensity (% browsed buds), leader browsing (% apical losses) and Relative Height Growth (RHG; year⁻¹). RHG data were log-transformed to fit a Gaussian distribution. The number of estimated parameters (K), AIC value and the difference between AIC value of a model and that of the most parsimonious (Δ AIC) are shown for each model. Likelihood ratio test support the results presented here

Model	Random effect	Fixed effects	K	AIC	Δ AIC
<i>Accumulated damage intensity</i>					
ACDI1	Pair	Species * Site	6	402.3	0.0
ACDI2	Pair	Species + Site	4	409.8	7.5
ACDI3	Pair	Species	2	410.4	8.1
ACDI4	Pair	Site	3	847.7	445.4
ACDI5	Pair	None	1	848	445.7
<i>Annual damage intensity</i>					
ANDI1	Pair	Species * Site	6	296.6	0.0
ANDI2	Pair	Species + Site	4	322.4	25.8
ANDI3	Pair	Species	2	319.2	22.6
ANDI4	Pair	Site	3	516.6	220
ANDI5	Pair	None	1	513.3	216.7
<i>Leader browsing</i>					
LB1	Pair	Species * Site	6	268.5	0.0
LB2	Pair	Species + Site	4	272.1	3.6
LB3	Pair	Species	2	278.2	9.7
LB4	Pair	Site	3	304.9	36.4
LB5	Pair	None	1	304.9	36.4
<i>Relative Height Growth</i>					
RHG1	Pair	Species * Site	6	-102.4	0.0
RHG2	Pair	Species + Site	4	-95.94	6.46
RHG3	Pair	Species	2	-67.15	35.25
RHG4	Pair	Site	3	-87.44	14.96
RHG5	Pair	None	1	-58.65	43.75

Accumulated and annual damage intensity was higher for *P. sylvestris* than for *P. nigra* in all the cases, as was leader browsing (Table 2 and 3; Fig. 2). There were differences among sites, as reported by the significant site factor and site * species interaction. Nevertheless, in all the cases the differences among two pines

were clear, with *P. sylvestris* showing consistently higher and often much higher damage than *P. nigra* (Table 2 and 3; Fig. 2).

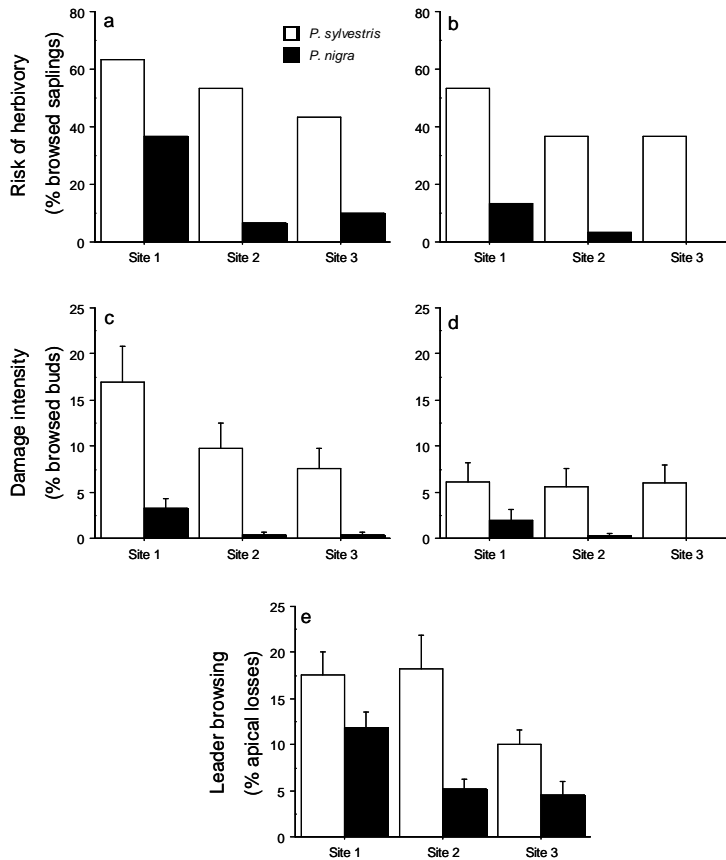


Figure 2 Accumulated (a) and annual (b) risk of herbivory represents the percentage of browsed saplings for *P. sylvestris* and *P. nigra* in the study sites taking in account browsing of the last three-four years and browsing of 2008, respectively. Accumulated (c) and annual (d) damage intensity show the percentage of browsed buds, and leader browsing (e) the percentage of apical losses since 1998. Bars indicate the standard errors of calculated means ($n = 30$)

Internode growth of the last four years standardized by internode age differed between browsed and unbrowsed saplings, pooling individuals of the three sites ($F = 12.98$, $P = 0.0005$ and $F = 35.31$, $P < 0.001$ for *P. sylvestris* and *P. nigra* saplings, respectively; Fig. 3). In addition, significant differences were found

between different years ($F = 2.72$, $P = 0.0494$ and $F = 4.39$, $P = 0.0064$ respectively).

Table 3 Fixed-effects estimates for each parameter included in the most parsimonious models of annual and accumulated damage intensity (% browsed buds) and leader browsing (% apical losses). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Parameter	Estimate	SE	z-value	P
<i>Accumulated damage intensity (ACDII)</i>				
Intercept	-5.1982	0.5282	-9.841	< 2e-16***
<i>P. sylvestris</i>	2.2087	0.1901	11.621	< 2e-16***
Site 2	-2.5023	0.8825	-2.835	0.00458**
Site 3	-2.3876	0.8902	-2.682	0.00732**
<i>P. sylvestris</i> : Site 2	1.3560	0.5084	2.667	0.00765**
<i>P. sylvestris</i> : Site 3	0.9623	0.5136	1.874	0.06100
<i>Annual damage intensity (ANDII)</i>				
Intercept	-6.0617	0.5838	-10.382	< 2e-16***
<i>P. sylvestris</i>	1.4446	0.2667	5.416	6.1e-08***
Site 2	-2.1536	0.9759	-2.207	0.02733*
Site 3	-16.5191	715.5196	-0.023	0.98158
<i>P. sylvestris</i> : Site 2	1.6893	0.5982	2.824	0.00474**
<i>P. sylvestris</i> : Site 3	16.2249	715.5192	0.023	0.98191
<i>Leader browsing (LBI)</i>				
Intercept	-2.1489	0.2127	-10.102	< 2e-16***
<i>P. sylvestris</i>	0.4847	0.2286	2.120	0.03396*
Site 2	-1.0001	0.3581	-2.793	0.00522**
Site 3	-1.0630	0.3680	-2.888	0.00387**
<i>P. sylvestris</i> : Site 2	1.0021	0.3759	2.666	0.00768**
<i>P. sylvestris</i> : Site 3	0.3818	0.4040	0.945	0.34463

INSECT HERBIVORY

The percentage of saplings affected by *Rhyacionia duplana* and *Thaumetopoea pityocampa* was very low for both species (Table 4). Although incidence of herbivory by *Retinia resiniella* (only for *P. sylvestris*) and leaf feeders was

considerable, both the number of galls and the percentage of damaged needles per sapling were rather low (Table 4).

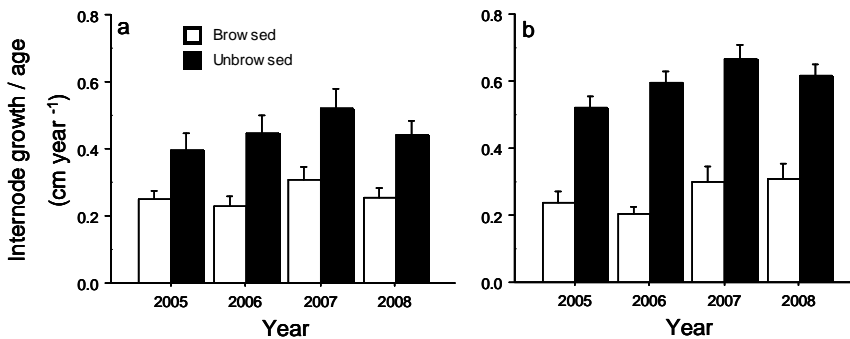


Figure 3 Differences between unbrowsed and browsed saplings for internode growth standardized by internode age (cm year⁻¹) for *P. sylvestris* (a) and *P. nigra* (b). Untransformed data are shown to allow easy interpretation. Bars indicate the standard errors of calculated means. *P. sylvestris*: unbrowsed, n = 44; browsed, n = 46. *P. nigra*: unbrowsed, n = 74; browsed, n = 16

DISCUSSION

The present work analyses the influence of herbivory in two coexisting pine species at the treeline. According to our first hypothesis, the impact of ungulates on pine saplings was higher than the impact of insect herbivores, which caused minor damage. As predicted by our second hypothesis, ungulates inflicted heavier damage in *P. sylvestris* than in *P. nigra*. Lower internode growth for browsed saplings reflects the retarded height growth that can result from ungulate browsing. Thus, ungulate herbivory appears to be the most harmful type during pre-reproductive years in the pine species studied. Although *R. resiniella* presented a high incidence in *P. sylvestris* saplings, the number of galls per saplings was low, and a gall did not always imply the breakage of the shoot or bud, which would translate as a loss of biomass. Leaf-feeder damage was recorded for a considerable number of

saplings for both species, but the percentage of needles affected was very low, and normally only some portions of the needles were damaged (authors' personal observation). By contrast, ungulate herbivory affected a higher number of saplings, inflicting considerable damage in browsed saplings, as indicated by the reduction in internode growth.

Table 4 Summary for insect herbivory incidence (percentage of affected saplings) and damage intensity for *P. sylvestris* and *P. nigra* saplings pooling the three sites (n = 90). In the case of *Retinia resiniella* the damage intensity was measured as number of galls per sapling, for leaf feeders as percentage of damaged needles, for *Rhyacionia duplana* as number of affected shoots, and for *Thaumetopoea pityocampa* as number of silk nests

Organism	Incidence	Damage intensity	SE	Range
<i>Retinia resiniella</i>				
<i>P. sylvestris</i>	61.11	2.01	0.29	16
<i>P. nigra</i>	1.11	0.05	-	-
Leaf feeders (<i>Achantolida</i> /<i>Diprion</i>)				
<i>P. sylvestris</i>	24.44	1.72	0.38	20
<i>P. nigra</i>	40	3.39	0.63	30
<i>Rhyacionia duplana</i>				
<i>P. sylvestris</i>	2.22	0.02	0.01	1
<i>P. nigra</i>	15.56	0.31	0.09	6
<i>Thaumetopoea pityocampa</i>				
<i>P. sylvestris</i>	2.22	0.02	0.01	1
<i>P. nigra</i>	8.89	0.11	0.04	3

Three different factors can explain the low incidence of insect herbivores found in this study: first, pine density was low at the treeline, which can hamper the movements of insects between trees (Dalin *et al.* 2009); second, abiotic conditions at the treeline are extreme, and, despite the trend of climatic change, temperature is still the prime factor influencing the physiology of insect pests (e.g. Bale *et al.* 2002); and third, for the case of *T. pityocampa*, our sampling coincided with a lower phase of incidence of this plague in S Spain (e.g. Hódar and Zamora 2009). In contrast, most of these factors do not affect ungulates. They are quite generalist, have a higher movement capacity, and select food in a hierarchical

fashion: plant communities at the landscape scale, feeding stations at the plant-community, and individual plants or plant parts at the bite scale (Senft *et al.* 1987; Provenza *et al.* 2002). Thus, ungulates foraging at the treeline find a landscape composed of stunted scrubs, most of them spiny and/or unpalatable, and scattered young pines attracting attention from this background, which concentrates all the attacks of the ungulates even taking into account the relatively low palatability of pines (Gómez *et al.* 2001). Taken all together, the result is a much higher herbivory intensity by ungulates than by insects.

It is clear from our results, that ungulates preferred *P. sylvestris* over *P. nigra*, in agreement to previous studies in the area (Baraza *et al.* 2009). This preference involves higher browsing damage for large number of *P. sylvestris* saplings, which consequently suffered a reduction in height growth, and therefore an increase in pine saplings risk of herbivore damage. In Mediterranean areas, *P. sylvestris* saplings presented low growth rates in comparison with northern latitudes (Danell *et al.* 1991; Väisänen *et al.* 1992; Edenius *et al.* 1995), thus aggravating the effects of herbivory. Furthermore, browsing in Mediterranean areas is more severe in summer when other food resources for ungulates are less abundant, leaving less time to recover from damages (Hester *et al.* 2004). All these factors exacerbate browsing damage, which reduces height growth, thereby increasing the time needed for saplings to achieve maturity (Zamora *et al.* 2001). *P. nigra* becomes sexually fertile later than *P. sylvestris*, but once mature, *P. nigra* effective fecundity is greater than that of *P. sylvestris* (Debain *et al.* 2005, 2007; Boulant *et al.* 2008). Therefore, the delay in reaching reproductive age for *P. sylvestris* due to ungulate browsing diminishes its competitiveness with respect to *P. nigra*. Finally, *P. nigra* showed higher or similar RHG than *P. sylvestris* at the treeline. Thus, at sites where *P. sylvestris* presented lower growth rates than *P. nigra*, higher browsing damages for *P. sylvestris* will accentuate the differences in performance and time of maturity between the two species.

Furthermore, several studies showed worse response to drought for *P. sylvestris* than for *P. nigra* both at the seedling and sapling stage (Castro *et al.* 2002, 2004), and at the adult stage (Martínez-Vilalta and Piñol 2002) in seasonal dry Mediterranean mountains. This vulnerability to drought is of special relevance under the prediction of increased frequency and severity of droughts in the Mediterranean Basin (Moreno 2005; FIC 2006; IPCC 2007). In fact, drought-induced mortality and decline symptoms have been observed in several *P. sylvestris* Mediterranean populations in recent years (Martínez-Vilalta and Piñol 2002; Thabeet *et al.* 2009; Galiano *et al.* 2010). Thus, *P. sylvestris* is the species most prone to suffer both drought- and ungulate-induced damages. This reinforcement of climatic responses by ungulate browsing can have major consequences for the dynamics of treelines in widespread *P. sylvestris*-*P. nigra* mixed forests. Under the scenario of increasing aridity and maintenance or increase of ungulate populations (due to the lack of predators and difficulties to perform adequate management; Cote *et al.* 2004), *P. sylvestris* upslope movements could be impeded. By contrast, the less herbivorized, more drought-tolerant *P. nigra* may have an opportunity for altitudinal expansion to other areas currently dominated by *P. sylvestris* (see Debain *et al.* 2005, 2007; Boulant *et al.* 2008).

In conclusion, our field sampling design enabled us to disentangle the effects of invertebrate and vertebrate herbivory in Mediterranean pine treelines formed by *P. sylvestris* and *P. nigra*. The present study demonstrates that saplings of both species at Mediterranean treelines suffer a weak invertebrate herbivore pressure, but a high, species-specific ungulate damage, concentrated on *P. sylvestris*. Thus, ungulate herbivory reinforce climatic response of coexisting *P. sylvestris* and *P. nigra*, and could favour a change in community dominance towards Mediterranean *P. nigra* at the upper altitudinal margin.

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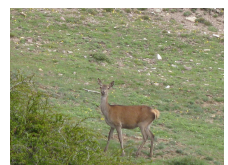
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CHAPTER 3:
HIERACHICAL MODELING OF LONG-TERM EFFECTS OF
CLIMATE AND HERBIVORY ON TREE SAPLING GROWTH:
A NEW APPROACH TO AN OLD PROBLEM

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ABSTRACT

Climate and herbivory are among the main factors influencing the distribution and abundance of plant species. Disentangling their relative importance in plant performance should thus be instrumental to forecasting plant species distributional changes. A long-term monitoring of weather fluctuations, pine saplings height growth, herbivory damages and ungulate population dynamics was performed in two woodland and treeline ecosystems of Southern Europe (SE Spain). A hierarchical Bayesian time series model was developed to estimate the joint effects of climate and herbivory on the temporal dynamics of the height growth of saplings from *Pinus sylvestris* and *P. nigra*. The ungulate population grew exponentially across time, which caused an increase in leader browsing in the studied plots. Both summer rainfall and herbivory were major factors determining multi-species height growth. For *P. sylvestris*, the impact of summer rainfall was much stronger than herbivory effects on plot-level height growth, despite the large browsing damages found in some individuals and plots. In particular, rainfall effects were larger in treelines than in woodlands, probably due to harsher environmental conditions in the former. In contrast, as a consequence of its greater drought tolerance, the relative height growth of the Mediterranean *P. nigra* was weakly affected by inter-annual variations in summer rainfall. Interestingly, herbivory simultaneously depressed relative height growth of both species in a single woodland plot. Therefore, drought sensitivity of the boreo-alpine *P. sylvestris* can be exacerbated by herbivory, which may have critical demographic consequences as height growth is closely related to maturity age.

Keywords: Bayesian analysis, hierarchical models, time series, height growth, herbivory, individual heterogeneity, long-term studies, Red deer, *Pinus* sp.

INTRODUCTION

Climate and herbivory, as bottom-up and top-down forces respectively, are among the main factors influencing the distribution and abundance of plant species (Davidson 1993, Archibold 1994, Speed et al. 2010). Experimental studies provide the basis for a mechanistic understanding of climate and/or herbivory effects in plant performance (e.g. Post and Pedersen 2008, Matías et al. 2011). However, they must be backed-up by observational studies performed at large-spatio-temporal scales in order to assess the relevance of these factors in nature. In this context, long time-series from wide geographical areas and heterogeneous habitats are an invaluable source of information to determine the relative importance of climate and herbivory in plant performance. Time-series analysis allows both the modeling of plant growth trends, and the assessments of the shifts in herbivory damages associated with climate and land-use changes (Cote et al. 2004, Wardle and Bardgett 2004). Therefore, long-term monitoring could help both to forecast future changes in plant species distribution in response to global change and in the design of management policies devoted to mitigating such changes (Lindenmayer and Likens 2009).

The need for accurate predictions of plant responses to local and global changes urges for the modeling of multiple sources of uncertainty (Cressie et al. 2009; Clark et al. 2011). In recent years, Bayesian hierarchical modeling (BHMs) arose as a flexible framework for structuring different sources of uncertainty and heterogeneity arising from the process, parameter and data levels of ecological models (Cressie et al. 2009). For example, recent applications of BHMs showed that differences between individuals within populations play an important role in species coexistence in forest ecosystems (Clark et al. 2007, 2010). Thus, it is essential to account for heterogeneity among individuals when analyzing the impact of climate and herbivory in demographic rates such plant growth. To this

end, the natural link of time-series analysis with BHM should help to disentangle the importance of climate and herbivory in plant distribution and performance.

At species distribution margins, where rapid distributional changes have been recorded in response to increasing warmer conditions (Parmesan and Yohe 2003, Peñuelas et al. 2007), interactions between abiotic and biotic factors play a critical role in these changes (Olofsson et al. 2009, Speed et al. 2010). Herbivory, through its influence in plant-community dynamics (Davidson 1993; Schmitz 2008), can either inhibit or exacerbate plant responses to climate-change conditions (Post and Pedersen 2008, Olofsson et al. 2009, Speed et al. 2010). Disentangling the relative importance of climate and herbivory in plant performance at range edges could thus help to forecast species distributional changes and clarify possible roles of abiotic-biotic interactions under global change scenario. There is an urgent need to analyze the effect of such interactions in water stressed areas such Mediterranean Basin, where big changes in plant-community composition are expected in response to drought and warming (Thuiller et al. 2005).

Here, we analyze tree saplings height growth response to climate and herbivory in Mediterranean pinewoods formed by *Pinus sylvestris* L. and *P. nigra* Arnold. We performed a long-term monitoring of weather fluctuations, saplings height growth, herbivory damages and number of ungulates during 16 years in two different valleys in Sierra de Baza (SE Spain), the absolute southernmost distribution for *P. sylvestris* (Boratynski 1991). The goal of the present study is to determine the relative importance of climate and herbivory in pine saplings performance of both species, which display differing ecological performance in response to environmental factors (Rouget et al. 2001; Martinez-Vilalta and Piñol 2002). Due to the repeated droughts and the increase of wild ungulate populations in the study area, we expect a positive effect of rainfall and negative impact of browsing in saplings height growth. Within each of the two monitored locations, we compare treeline and woodland areas to test if pine saplings display different response to climate and herbivory in its altitudinal margin. We expect higher

rainfall effect in treeline due to harsh environmental conditions. Summarizing, the specific objectives of this study are: 1) to test the effect of precipitation and herbivory in height growth trends; and 2) to compare the effect of both factors in treeline and woodland areas.

MATERIAL AND METHODS

STUDY SITE AND UNGULATE POPULATIONS

The study was conducted at Sierra de Baza Natural Park (SE Spain, 2°51'48''W, 37°22'57''N). Sierra de Baza, together with Sierra Nevada, represents the absolute southernmost distribution limit for *Pinus sylvestris* (Boratynski 1991). The climate is Mediterranean, characterized by cold winters and hot summers, with pronounced summer drought (June-August). Precipitation is concentrated mainly in autumn and spring. The annual rainfall is 495 ± 33 mm (mean \pm SE for 1991-2006 period), and summer rainfall (months of June, July and August) is 31 ± 9 (mean \pm SE for 1991-2006 period; Cortijo Narváez meteorological station, 1360 m a.s.l.). The bedrock is predominantly calcareous.

Both wild and domestic ungulates are present in Sierra de Baza. Among the wild ungulate species, the regional red deer (*Cervus elaphus* L.) population increased from only 81 individuals introduced in the Natural Park in 1988 to more than 1800 in year 2000, due to the absence of top predators (Granados et al. 2001). From 2005 to 2007 the red deer population was monitored through stratified lineal transects following the same procedure as Granados et al. (2001), which yielded an approximate population of 2600 individuals in 2007 (5.04 ind/km²; CMA 2008). In contrast, the Spanish ibex (*Capra pyrenaica* Schinz) is present at the study area at very low population densities (0.39 individuals km⁻²; CMA 2009). With respect to domestic ungulates, from May to December herds of domestic goat (*Capra*

aegagrus hyrcus L.) and sheep (*Ovis aries* L.) increase notably the grazing pressure, with a density of 40 ind/km² (CMA 2008). For the purpose of this study only the red deer population will be considered, as domestic ungulate pressure remained fairly constant during the last 16 years (CMA 2008) and the population of Spanish ibex is very small.

SAMPLING DESIGN, UNGULATE BROWSING, AND PLANT PERFORMANCE

This study was conducted in two native forests in Sierra de Baza from 1993 to 2008. In each forest we selected two plots (approximately 1 ha surface each), one in the woodland and the other at the treeline. The first forest, Boleta hereafter, is a mixed forest (1700-2100 m a.s.l.) of *P. sylvestris* and *P. nigra* growing intermingled with an understory composed mainly of *Juniperus communis* L., *J. sabina* L., *Berberis hispanica* L., *Astragalus granatensis* Lange and *Hormatophylla spinosa* (L.) P. K pfer. *P. sylvestris* sapling density was 142 ± 24.3 and 72 ± 12.7 ind/ha in woodland and treeline respectively; and *P. nigra* density was 88 ± 34.3 and 2 ± 2 ind/ha. Pine sapling density was measured with 10 transects of 50 m length and 10 m wide at each plot in 2008. The second forest, Fonfr a hereafter, is composed exclusively of *P. sylvestris* with an understory composed mainly by *J. communis* and *J. sabina*. Sapling density was 576 ± 91.8 and 142 ± 35.1 ind/ha for woodland and treeline respectively. Overall, we sampled 234 pine saplings of *P. sylvestris* and *P. nigra*: 90 *P. sylvestris* and 55 *P. nigra* in Boleta woodland, 32 *P. sylvestris* in Boleta treeline, 31 *P. sylvestris* in Fonfr a woodland, and 26 *P. sylvestris* in Fonfr a treeline. Only 5 pine saplings of *P. nigra* were sampled at Boleta treeline, so we discarded them for the analysis. Monitored individuals were established non-reproductive saplings (older than 3 years old) at the beginning of the study. The final number of sampled saplings in each plot varied in part because

of field sampling problems such as destruction of tags and other signals by ungulates.

Leader browsing, which causes the loss of apical dominance, was monitored from 1993 to 2008 as percentage of sampled saplings which suffered a loss of apical dominance. Age was estimated as number of whorls for each sapling (Edenius et al. 1995). Annual height for the period 1993-2008 was also measured and used to calculate annual Relative Height Growth (hereafter RHG, Leopold and Kriedemann 1975; see: Model construction in Hierarchical time series modeling of climate and herbivory effects).

HIERARCHICAL TIME SERIES MODELLING OF CLIMATE AND HERBIVORY EFFECTS

MODEL CONSTRUCTION

We constructed a hierarchical Bayesian time series model (hereafter HBTS model; Berliner 1996) to estimate the joint effects of climate and herbivory on the temporal dynamics of RHG on several levels of ecological organization. Our approach is based on the simultaneous estimation of two separate models, one for the stochastic dynamics of the red deer population at the landscape level, and the other for the environmental forcing of the RHG at the individual, plot and species levels (see Fig. 1). At the upper level, the dynamics of the red deer population was modeled with a state space approach (Clark & Bjørnstad 2004). In a state space model, the time series of observed population estimates is linked to the true (latent) abundances through a measurement equation, while the time series of the latent values is modeled through an ecological process model. We used a Gompertz

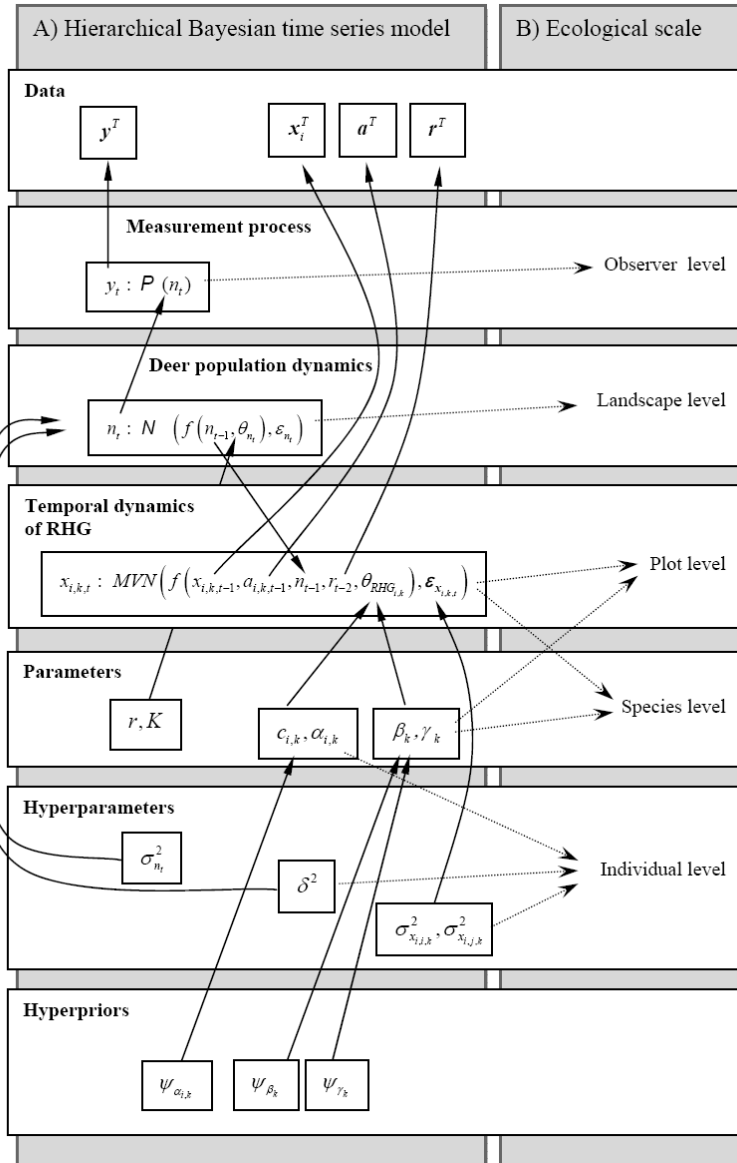


Figure 1 A graphical representation of the Hierarchical Bayesian time-series model (HBTS) linking ecological processes, observed data and model parameters in a structured framework. A) The solid arrows link the different sub-components used for jointly modeling the population dynamics of red deer and the long-term effects of herbivory and climate on the relative height growth of pines, while accounting for allometric growth; these sub-components are further linked to different levels of ecological organization (B) with dotted arrows. Every process and parameter naturally emerges from at least one scale of this organization, thus structuring uncertainty and variability in a hierarchical manner. Note that the eqns. 1.3 are represented in this figure as normal and multivariate normal probability distributions for the red deer and relative height growth (RHG) dynamics, respectively. The vectors θ_{n_t} and $\theta_{RHG_{i,k}}$ encompass the set of parameters for the red deer and RHG process equations, respectively. See Hierarchical time series modeling of climate and herbivory effects for a description of the rest of parameters and variables.

kernel (Dennis and Taper 1994) to model the evolution of the true population of red deer across the landscape,

$$n_t = n_{t-1} + r \left(1 - \frac{n_{t-1}}{K} \right) + \varepsilon_t$$

eqn. 1

where n_t is the \log_e -transformed true population size at time t , r is the intrinsic rate of increase, K the carrying capacity at the landscape level and ε_t is the stochastic term for the environmental and demographic process error. The vector $\varepsilon_t = (\varepsilon_{1,t}, \dots, \varepsilon_{S,t})^T$ is sequentially independent noise distributed according to a normal distribution with mean 0 and variance Σ_{n_t} , $\varepsilon_t \sim N(0, \Sigma_{n_t})$. The matrix Σ_{n_t} is decomposed into an environmental ($\sigma_{n_t}^2$) and demographic component (\mathbf{D}_t), $\Sigma_{n_t} = \sigma_{n_t}^2 + \mathbf{D}_t$, where $\sigma_{n_t}^2$ stand for the impact of environmental noise and \mathbf{D}_t for the impact of demographic stochasticity. The diagonal matrix $\mathbf{D}_t = [\delta^2/\exp(n_1), \dots, \delta^2/\exp(n_S)]^T$ reflects the demographic variance affecting the dynamics of the red deer from time $t-1$ to t , which scales inversely with population size (e.g., Engen et al. 1998). Finally, the population estimates are linked to the true abundances through a Poisson observation model,

$$y_t \sim Poi(n_t)$$

eqn. 2

where y_t is the observed red deer abundance at time t . A Poisson distribution is suitable in our case because the survey at the landscape level yielded population counts, and in this setting all of the parameters in the state space model are statistically identifiable (Knappe et al. 2011).

In a second level, the HBTS model estimates the environmental forcing of the temporal dynamics of RHG, as exerted by climate and herbivory, along with

the constraint imposed by the allometric relationship between tree growth and age (Fig. 1). While we are interested in estimating environmental effects at both the species and plot levels, we included allometric terms at the individual level due the heterogeneity in age-structure of our tree sample. We constructed separate models for *P. nigra* and *P. sylvestris*. Be $x_{i,k,t}$ the \log_e -size of the individual pine i located in the k plot at time t , and be $a_{i,k,t}$ the \log_e -age of this pine; we can thus construct the $T \times n$ matrix $\mathbf{x}_{k,t}$ and $\mathbf{a}_{k,t}$ including the individual temporal contributions to size- and age-structure of each plot, respectively (note that T is the length of the time series and n is the number of individual pines). If we further denote the time series for summer rainfall at the study area by $r_t = [r_{t-T}, r_{t-T+1}, \dots, r_t]^T$, the dynamics of the RHG can be modeled as

$$\mathbf{x}_{k,t} = \mathbf{x}_{k,t-1} + \mathbf{c}_k + \mathbf{a}_k \mathbf{a}_{k,t-1} + \beta_k n_{t-1} + \gamma_k r_{t-2} + \boldsymbol{\varepsilon}_t$$

eqn. 3

where $x_{i,k,t} - x_{i,k,t-1}$ is the standard RHG measure for each pine (Leopold and Kriedemann 1975). The vector \mathbf{c}_k contains the individual constant-level parameters, $c_{i,k}$. The parameter vector \mathbf{a}_k contains the individual-level coefficients expressing the allometric relationship between size and age for each pine, $\alpha_{i,k}$; previous analysis (not shown) suggested simple log-linear functions as the best functional forms expressing these relationships. The parameter β_k measures the effect of ungulate density on RHG at the plot level, where n_{t-1} is the latent population density of red deer at the landscape level estimated on-line through the state-space specification in eqns. 1-2 above; the term γ_k measures the lagged effect of summer rainfall on RHG at the plot level. We used data on summer rainfall because the effect size during these months was larger than the effect of annual rainfall (data not shown). Finally, $\boldsymbol{\varepsilon}_t$ is the term for environmental noise, distributed according to a multivariate normal distribution with 0 mean and a variance-covariance matrix $\Sigma_{\mathbf{x}_{k,t}}$, $\boldsymbol{\varepsilon}_t \sim \text{MVN}(0, \Sigma_{\mathbf{x}_{k,t}})$. The matrix $\Sigma_{\mathbf{x}_{k,t}}$ has the form

$$\Sigma_{\mathbf{x}_{k,t}} = \begin{pmatrix} \sigma_{x_{k,1,1}}^2 & \sigma_{x_{k,1,2}}^2 & L & \sigma_{x_{k,1,j=n}}^2 \\ \sigma_{x_{k,2,1}}^2 & \sigma_{x_{k,2,2}}^2 & L & L \\ M & M & O & M \\ \sigma_{x_{k,j=n,1}}^2 & L & L & \sigma_{x_{k,j=n,j=n}}^2 \end{pmatrix}$$

eqn. 4

where the terms in the main diagonal represent the impact of environmental stochasticity on the temporal trajectory of the RHG of each pine; the covariance terms in the off-diagonal model the impact of environmental stochasticity on the cross-correlated (joint) stochastic trajectories among individual pines. That is, the terms $\sigma_{x_{k,i,j}}^2$ measure the synchronized responses of each pair of pines to latent (unmeasured) environmental fluctuations.

Overall, note that the model expressed in eqn. 3 is a linear mixed-effects model including fixed effects for climate and herbivory at the plot level, random individual effects for allometric tree growth, random (individual-level) intercepts, and individual-level covariation in the joint responses to stochastic factors among every pair of pines. Besides, the state-space Gompertz approach used for modeling the dynamics of red deer at the landscape level (eqns. 1 and 2) is identical to a repeated-measures analysis of variance model with a random time effect (e.g., Dennis et al. 2006), further accommodating non-Gaussian random error distributions. Seeking for generalization, this places our HBTS model within the growing framework of Hierarchical ecological modeling approaches (e.g., Royle & Dorazio 2008).

PRIOR SPECIFICATION AND PARAMETER ESTIMATION

We fitted the HBTS model using Markov Chain Monte Carlo (MCMC) integration through Gibbs sampling (King et al. 2010). To let the likelihood dominate the prior, we placed weakly informative prior distributions on the unknown quantities in eqns. 1-3. Proper uniform distributions were placed on the standard deviations of the environmental and demographic noise terms in the Gompertz state-space model, $\sigma_n^2, \delta^2 \sim Unif(0, 10)$ (See Gelman 2006). The variance-covariance matrix $\Sigma_{\mathbf{x}_{k,t}}$ was modeled with an inverse Wishart prior, $[\Sigma_{\mathbf{x}_{k,t}}]^{-1} \sim \text{Wishart}(\mathbf{\Omega}, S)$, which is the conjugate prior for the covariance matrix of a multivariate normal distribution (Gelman et al. 2004). The number of degrees of freedom was set to the rank of the scale matrix $\mathbf{\Omega}$, namely the number of trees per plot (n); this is the value expressing the weakest prior information. The location parameters in eqns. 1-2 and the constant level terms in eqn. 3 ($c_{i,k}$) were given flat (extremely platykurtic) prior normal distributions, $r, K, c_{i,k} \sim N(0, 10^6)$. Finally, the parameters measuring the effects of ungulates, climate and allometric growth were given normal distribution with Gaussian mixture variance hyperpriors, $\beta_k \sim N(0, \psi_{\beta_k}), \gamma_k \sim N(0, \psi_{\gamma_k}),$ and $\alpha_{i,k} \sim N(0, \psi_{\alpha_{i,k}})$, respectively (see: *Assessing the evidence for herbivory and climatic effects*).

We programmed the HBTS model in the BUGS language and used WinBUGS 1.4.1 (Spiegelhalter et al. 2003) to fit the model. We ran three independent Markov chains with dispersed initial values for 100,000 iterations, and discarded the first 50,000 as a burn-in period. To increase the precision of Bayesian estimates, we merged the unthinned chains (Link & Eaton, *in press*). We used the R package BOA (Smith 2007; R Development Core Team 2011) to derive the posterior estimates of parameters and latent states, and to check the convergence of the chains using standard diagnostic tests (see Gelman et al. 2004). Finally, we

tested whether the residual variance-covariance matrix $\Sigma_{x_{k,t}}$ conformed to the multivariate normality assumption using the Shapiro-Wilks test (Mutshinda et al. 2011).

ASSESSING THE EVIDENCE FOR HERBIVORY AND CLIMATIC EFFECTS

In order to assess the statistical evidence in favor of the inclusion of a given environmental or allometric effect in the HBTS model, we followed O'Hara and Sillanpää (2009) and used Stochastic Search Variable Selection (SSVS) to automatically set close to 0 those location parameters in eqn. 3 ($\alpha_{i,k}$, β_k , γ_k) that are found statistically spurious during the MCMC simulation. Taking advantage of the Gibbs sampler (George and McCulloch 1993), the SSVS method allows the embedding of the HBTS model in a hierarchical normal mixture model in which a set of latent indicators can identify candidate predictor variables (O'Hara and Sillanpää 2009). In this setting, a variance hyperprior (denoted by ψ_{\cdot}) is included in each prior distribution for the effects of ungulates, climate and allometric growth, $\beta_k \sim N(0, \psi_{\beta_k})$, $\gamma_k \sim N(0, \psi_{\gamma_k})$, and $\alpha_{i,k} \sim N(0, \psi_{\alpha_{i,k}})$, respectively. The terms ψ_{\cdot} are specified so that they are small when the estimated effect is small and large when the estimated effect is far from 0. Specifically, we can define an auxiliary binary indicator g modelled through a Bernoulli distribution, $g \sim \text{Bern}(p)$, where $0 < p < 1$, so that $g = 1$ when the parameter is included in the model and 0 otherwise. Conditional on this indicator, the prior distribution of the unknown parameter is thus the mixture of two Gaussians, specifically: $\alpha_{i,k}, \beta_k, \gamma_k \mid g \sim (1 - g) \times N(0, q_1) + g \times N(0, q_2)$; alternatively, $\psi_{\cdot} = (1 - g) \times q_1 + g \times q_2$. In our setting, we set $q_1 = 10^{-5}$, $q_2 = 10$ and $p = 0.5$ (different values yielded similar results). Thus, if the effect of a given parameter on RHG is large, the binary indicator g will most often be around

1 and so the variance ψ will be around 10; in this case the parameter is estimated freely with a rather flat prior distribution (namely, the spike of the Gaussian mixture). In contrast, if the effect of a the parameter is small, the binary indicator g will most often be around 0 and so the variance ψ will be around 10^{-5} (the slab of the Gaussian mixture); in this situation the parameter is restricted to be effectively 0, so its estimation does not affect the model results (see O'Hara and Sillanpää (2009) for further details and Mutshinda et al. (2011) for an ecological example).

One key advantage of SSVS is that the calculation of Bayes Factors (BF) is straightforward. As a Bayesian version of hypothesis testing (Kass & Raftery 1995; Gelman et al. 2004), the BF quantifies the amount by which the prior odds of including vs. omitting a given effect ($\alpha_{i,k}$, β_k , γ_k) is changed into posterior odds by the data. That is,

$$BF_{\beta_k, \gamma_k, \alpha_{i,k}} = \frac{P(g = 1 | \text{Data})}{1 - P(g = 1 | \text{Data})} \times \frac{1 - P(g = 1)}{P(g = 1)}$$

eqn. 5

where $P(g = 1 | \text{Data})$ is the posterior odds and $P(g = 1)$ is the prior odds. Since the prior odds of including a given interaction was set at $p = P(g = 1) = 0.5$ in our case, the BF is simply the ratio of two likelihoods, one for the hypothesis of parameter inclusion in the model (H_1) and the other for the hypothesis of parameter exclusion (H_2). Following Jeffreys' (1961) scale, we interpret the value of the BF in the following way: $BF_{H_1 \text{ vs. } H_2} < 1$, Negative support for the inclusion of the parameter in the HBTS model (H_1); $1 \leq BF_{H_1 \text{ vs. } H_2} < 3$, Barely worth mentioning evidence in favor of H_1 ; $3 \leq BF_{H_1 \text{ vs. } H_2} < 10$, Substantial support for H_1 ; $10 \leq BF_{H_1 \text{ vs. } H_2} < 30$, Strong support for H_1 ; $30 \leq BF_{H_1 \text{ vs. } H_2} < 100$, Very strong support for H_1 ; $BF_{H_1 \text{ vs. } H_2} > 100$, Decisive support for H_1 .

RESULTS

The diagnostic tests revealed good mixing of the MCMC chains and rejected the hypothesis of non-convergence. Additionally, the Shapiro-Wilk's test suggested that the residual variance-covariance matrix conformed to the multivariate normality assumption ($P > 0.05$).

POPULATION DYNAMICS OF RED DEER

The carrying capacity of the red deer population at the landscape level (parameter K in eqn. 1) was estimated at 2912 individuals (MC standard error of the posterior mean: 891), and the intrinsic growth rate (r) at 0.388 ± 0.173 . According to the fitting of the state-space Gompertz population dynamics model, the 71.271% of the temporal variability in red deer abundance was driven by environmental stochastic effects, while intra-specific interactions (density dependence) accounted only for the 28.684% of this variance. Since the relative impact of demographic stochasticity was negligible (0.045%), these results indicate that the red deer population increased in a density independent fashion across the landscape (See Fig 2A).

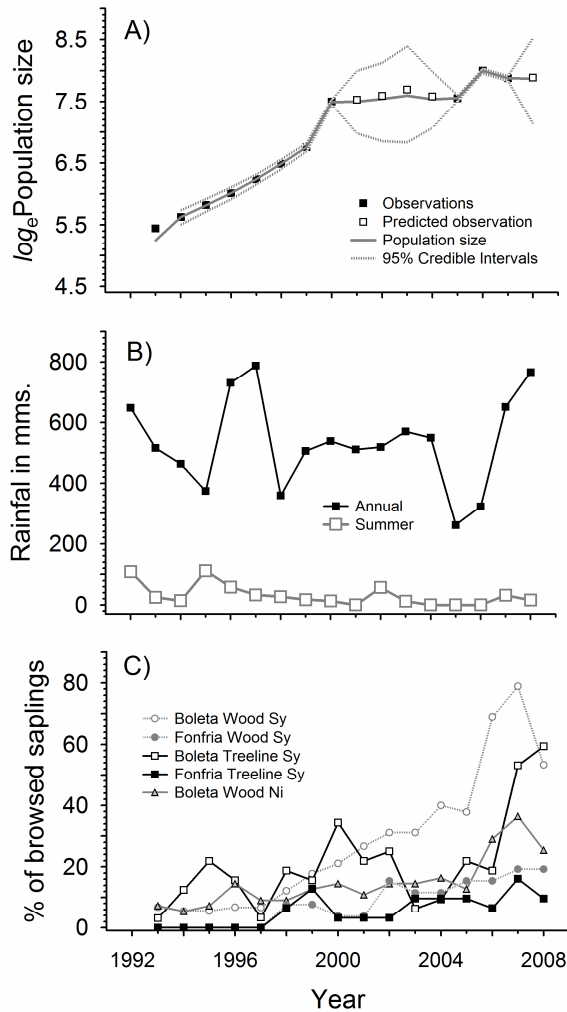


Figure 2 Time series plots of ungulate population size, rainfall variability, and herbivory intensity in the study area from 1992 to 2008. A) The observed yearly abundance (counts) of red deer abundance across the landscape is shown as black solid squares; the latent (unobserved) states estimated with the Gompertz state-space model is shown as a grey solid line, with 95% credible intervals depicted as grey dotted line; the estimated observations are shown as open squares. B) Time series of yearly rainfall (black squares) and rainfall during summer (June/July/August; grey open squares). C) Temporal evolution of the proportion of browsed saplings for each plot, spatial unit and pine species.

Table 1 Results of the fitting of the Hierarchical Bayesian time-series model to the dataset of relative height growth of pines from 1992 to 2008 across different plots, spatial units and species. Shown are the Bayesian posterior estimates (and 95% Credible Intervals) along with the Bayes Factors for these estimates.

Plot	Spatial unit	Species	Posterior estimates		Bayes Factors*		
			β (95% CI)	γ (95% CI)	α	β	γ
Fonfria	Treeline	<i>Pinus sylvestris</i>	-0.004 (-0.009, 0.001)	0.026 (0.022, 0.031)	0.002 (0, 0.098)	0.012	3996
Fonfria	Woodland	<i>Pinus sylvestris</i>	-0.004 (-0.008, 0.002)	0.020 (0.015, 0.025)	0.004 (0, 1.448)	0.008	796
Boleta	Treeline	<i>Pinus sylvestris</i>	-0.005 (-0.009, -0.001)	0.027 (0.022, 0.032)	0.007 (0, 1.124)	0.016	3996
Boleta	Woodland	<i>Pinus sylvestris</i>	-0.013 (-0.021, -0.008)	0.018 (0.015, 0.022)	0.004 (0, 1.996)	5.569	996
Boleta	Woodland	<i>Pinus nigra</i>	-0.015 (-0.023, -0.006)	0.012 (0.007, 0.019)	0.006 (0, 0.368)	7.594	2.462

Note: Parameter β stand for herbivory effects, while γ stand for climate effects. Those posterior estimates with Credible Intervals non overlapping 0 are shown in bold type.

* The Bayes Factors (BF) are shown for herbivory effects, climate forcing, and interindividual allometric growth, measured through parameter α in eqn. 3. In this latter case, shown are the median value across individuals within each site and spatial unit (plus minimum and maximum values across pines). For climate forcing and herbivory effects BF values were interpreted according to Jeffreys' (1961) scale: BF < 1, Negative support for the inclusion of the parameter in the HBTS model (H_1); 1 ≤ BF < 3, Barely worth mentioning evidence in favor of H_1 ; 3 ≤ BF < 10, Substantial support for H_1 ; 10 ≤ BF < 30, Strong support for H_1 ; 30 ≤ BF < 100, Very strong support for H_1 ; BF > 100, Decisive support for H_1 .

LONG-TERM EFFECTS OF HERBIVORY AND CLIMATE

The amount of precipitation during summer decreased significantly throughout the study period (Fig. 2B; Spearman rank's order correlation: $r_s = -0.55$, bootstrapped P-value: $P_{boot} = 0.012$, calculated using 10000 MC samples). In contrast, herbivory intensity, measured through the % of browsed saplings in the leader shoot increased across time in all plots (Fig. 2C). However, there was large inter-plot variability in herbivory intensity, with very high values in Boleta woodland (up to 80% of browsed saplings) and relatively low values in Fonfria treeline (< 20%).

Table 1 shows the posterior parameter estimates of the climate and herbivory effects on tree sapling growth, along with the estimated BF of each effect. After controlling for allometric growth, there was evidence for a negative impact of ungulate density on tree growth in Boleta (since parameter β does not overlap 0) but not in Fonfria; however, the BFs suggest that there is substantial support for this effect in the woodland of Boleta for both *P. sylvestris* and *P. nigra*, but a negative support (BF=0.016) for the inclusion of the parameter in the treeline. In contrast, the positive effect of rainfall on tree sapling growth (parameter γ) is supported in all plots and spatial units, particularly for *P. sylvestris*; the evidence is greatest in the treeline (BF=3996). There is decisive support for the inclusion of a rainfall effect in these cases. In contrast, there is only barely worth mentioning evidence in favor of a rainfall effect on *P. nigra* growth (BF=2.462). The statistical evidence for the inclusion of allometric effects (parameters α) is negative in all cases, with BFs from 0 to 1.996 (Table 1).

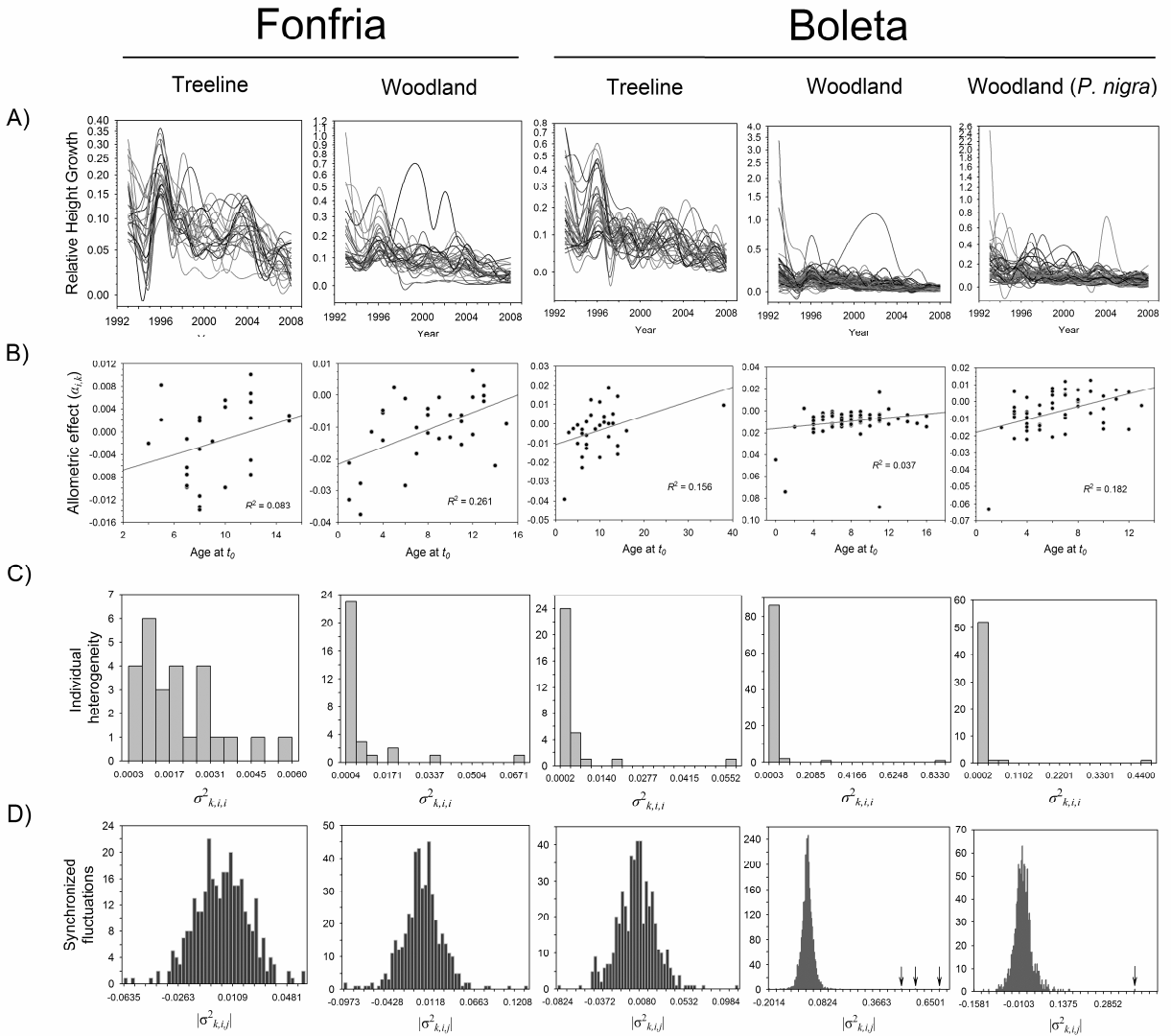


Figure 3 Structure of heterogeneity and stochastic effects estimated with the Hierarchical Bayesian time-series model across plots, spatial units and species. A) Time-series plots of the yearly relative height growth values for every individual pine and treatment. Time series were smoothed with a spline constructed placing a knot in every year and colored with differing shades of grey to ease visualization. Note the log-scale in the Y-axis. B) Regression of the allometric effect of age for individual pines ($\alpha_{i,k}$) on the initial age (Age at t_0). In all cases, the parameters $\alpha_{i,k}$ overlapped 0. C) Distribution of the marginal posteriors for the stochastic environmental effects at the individual level ($\sigma_{x_{k,i,j}}^2$). D) Distribution of the marginal posteriors for the synchronized responses of each pair of pines to latent (unmeasured) environmental fluctuations (Denoted as scaled covariance, $|\sigma_{x_{k,i,j}}^2|$, referring to correlations). The arrows indicate extreme values for these posteriors.

STRUCTURE OF HETEROGENEITY AND STOCHASTIC EFFECTS

Figure 3 shows the individual variability in tree RHG, the allometric relationships within each plot and the individual and synchronized structure of the stochastic environmental effects estimated with the HBTS model. The interindividual difference in the temporal growth trajectories is smaller in the woodland of Boleta relative to other sites and spatial units (Fig. 3A). As expected, the relationship between the allometric effect on sapling growth and the initial age of the sampled individuals is positive across all sites and spatial units (Fig. 3B), albeit this effect is generally weak and non-significant. There is substantial heterogeneity in the stochastic environmental effects on individual RHGs across all plots and spatial units (Fig. 3C), with non-Gaussian distributions of the marginal posteriors of individual effects in all cases (Shapiro-Wilk's test, $P < 0.05$). Finally, the synchronized responses of each pair of pines to latent environmental fluctuations were very weak across all plots and spatial units (Fig. 3D), with the Gaussian distributions of marginal posteriors centered around 0 (Shapiro-Wilk's test, $P < 0.05$). None of the potential synchronized trajectories were significant, which suggest that the temporal responses of individual pines to unmeasured factors were spatially decoupled across the landscape.

DISCUSSION

Assessing the response of individual organism to ecological heterogeneity is fundamental for understanding many ecological processes, such as interspecific resource partitioning, species coexistence, and population invasibility (Clark 2010). Here, we have developed a hierarchical Bayesian time-series model that simultaneously structure the demographic response to ecological heterogeneity at the individual, the species, and the plot levels. Specifically, the model accounted

for inter-individual variability in the allometric growth while jointly modeling the long-term impacts of summer rainfall and herbivory in height growth at the species and plot levels. For *P. sylvestris*, the model showed prevailing summer rainfall effect over herbivory in height growth, and this effect was larger in the treelines than in the woodlands. In contrast, the height growth of *P. nigra* displayed a limited influence of summer rainfall. On the other hand, herbivory affects negatively height growth of both species in a single woodland plot, although its effect in *P. sylvestris* was not as strong as the effect of summer rainfall. A substantial heterogeneity in the stochastic environmental impacts on individual height growth trends, as well as generalized decoupled responses of each pair of pines to other unmeasured stochastic factors, was detected across all plots and spatial units. The shoot growth of both *P. sylvestris* and *P. nigra* is mainly affected by environmental conditions during the period of bud formation (Dobbertin et al. 2010, Isik 1990); this provides an ecological mechanism for the 1-year lagged effect of summer rainfall on height growth. Leader browsing, which causes the loss of apical dominance, reduce height growth and can alter pine sapling morphology when occurring repeatedly (Gill 2006).

In addition to up-scaling heterogeneity from the individual to the plot level, our model allowed the simultaneous modeling of two different processes, such the red deer population and saplings height growth temporal dynamics. The results showed that red deer abundance was driven by environmental stochastic effects, increasing in a density independent fashion across the landscape. Warmer winters, absence of predators, increases of forage due to silvicultural activities, disproportionate male hunting and difficulties to adequately implement management policies seems to be the main reasons behind the observed increase of red deer population (Côte et al. 2004, CMA 2008). The increase in deer abundance, which seems to be a global phenomenon (Côte et al. 2004, Wardle and Bardgett 2004), prompted an increase in the percentage of saplings suffering a loss of apical dominance. The repeated leader browsing suffered by many saplings could

ultimately collapse forest regeneration induced by a severe reduction of height growth and morphological changes (Zamora et al. 2001, Côte et al. 2004). However, the effect of summer rainfall prevailed over herbivory in *P. sylvestris* height growth in the two monitored forests. Thus, for *P. sylvestris*, water availability is relatively more important than herbivory even in highly browsed bank of recruits.

The quantification of support for parameter inclusion through Bayes factors (BF) allows us to disentangle the relative statistical importance of the modeled effects. Herbivory negatively impacted height growth of *P. sylvestris* and *P. nigra* in one woodland plot. Nevertheless, the herbivory effect received substantial support according to BF for its inclusion in the model for *P. sylvestris*, in comparison with decisive support for summer rainfall. In addition, BF allows us to compare the importance of these parameters among different areas, such as summer precipitation in woodland and treeline areas for *P. sylvestris*. Although rainfall received in both areas decisive support for inclusion on the model, the values were much higher in the treelines. This could be due to harsher environmental conditions (low temperature, high wind and radiation exposition) suffered by saplings in the treeline. In an increased aridity scenario, where upward plant migrations have been already observed (Parmesan and Yohe 2003, Peñuelas et al. 2007), larger effects of summer rainfall in treelines could restrict these movements mediated by severe droughts. This would be especially so in southern ranges of species distributions and seasonally dry areas such as the Mediterranean basin.

In contrast to *P. sylvestris*, the effect of summer rainfall on *P. nigra* height growth was relatively weak. There was only a barely worth mentioning evidence in favor of a rainfall effect on *P. nigra* height growth in comparison with a decisive support for *P. sylvestris*. This result agrees with the studies showing higher sensitivity and vulnerability to drought for *P. sylvestris* relative to *P. nigra* during tree ontogeny (Castro et al. 2002, Martínez-Vilalta and Piñol 2002, Boulant et al. 2008). In contrast to the Mediterranean *P. nigra*, *P. sylvestris* is a boreo-alpine

species strongly affected by drought in its southern distribution range (Bigler et al. 2006, Galiano et al. 2010). In fact, drought-induced mortality and decline symptoms have already been observed in several *P. sylvestris* Mediterranean populations during the last years (Martínez-Vilalta and Pinol 2002, Galiano et al. 2010). Thus, the predicted decrease in summer rainfall by up to 50 % for 2100 in the Mediterranean basin, along with the predicted increase in summer temperature in 4-5 °C (IPCC 2007) could severely impact *P. sylvestris* growth. Reich and Oleksyn (2008) suggest that moisture stress enhanced by warming could lead to a future decrease in *P. sylvestris* height growth in central and southern Europe. Therefore, the lower effect of water availability during summer could provide a demographic advantage for *P. nigra* in an increased aridity scenario.

Herbivory impacted negatively both species in one of the woodland plots. However, in this plot, while *P. nigra* was weakly affected by summer rainfall, *P. sylvestris* was greatly impacted upon by this effect. Thus, the already mentioned drought sensitivity of *P. sylvestris* was exacerbated by herbivory, which could have critical demographic consequences since height growth is closely related to maturity age (Zamora et al. 2001 and references within). Therefore, under increasing aridity scenarios and unmanaged ungulate populations, the regeneration of *P. sylvestris* can be effectively suppressed, or at least slowed down, by severe reductions in height growth rate. In addition, an expected upward migration could be impeded by higher effect of water availability in treelines for *P. sylvestris*. By contrast, the more drought tolerant *P. nigra* may have an opportunity for altitudinal migration or expansion to other areas currently dominated by *P. sylvestris* (see Debain et al. 2007, Boulant et al. 2008).

In addition to the effects of summer rainfall and herbivory on height growth, we also expected significant individual heterogeneity in the allometric effects, as relative height growth diminishes with size and over time (Burdon and Sweet 1976); however the evidence for its inclusion in the model was negative. Summer rainfall and herbivory might affect this result in two non-exclusive ways.

Firstly, summer rainfall could disrupt growth differences due to age or size, triggering an increase in growth during wet years and a decrease during dry years across different age or size classes. Secondly, repeatedly browsed cushion-shaped saplings might display growth rate patterns similar to younger saplings of an analogous short height. Thus, despite a greater effect of rainfall in height growth, herbivory might influence individual allometric relationships through a strong phenotypic effect, acting as rejuvenating force. In fact, several grafting studies showed that old-aged apical meristems could return to juvenile conditions (Mencuccini et al. 2007). Therefore, it is possible that summer rainfall and herbivory are able to jointly modulate individual allometric effects; however further studies (e.g. analysis of allometric effects in combinations with fenced and irrigated saplings) could shed light on this.

In conclusion, our results showed the overwhelming importance of summer rainfall relative to herbivory in height growth, a critical demographic rate for forest regeneration and expansion. Disentangling the effects of climate and biotic factors and checking for interactions between them should be critical to improve predictions on ecosystem responses to global change. The effect of summer precipitation on height growth observed in this study agrees with the expected adverse impact of drought in plant species distribution and performance in the Mediterranean basin (Thuiller et al. 2005), especially for those situated at its distributional limit such *P. sylvestris*. Nevertheless, interactions with herbivory as recorded for *P. sylvestris* in woodland could hasten some expected changes (e.g., Post and Pedersen 2008). In addition, detecting which areas would be more vulnerable to drought, as it was found for the treelines in the present study, could improve our understanding of forest dynamics under current and future climate change scenarios. This study adds to recent approaches (e.g., Cressie et al. 2009; Clark 2010; Mutshinda et al. 2011) suggesting that the use of hierarchical modeling would be essential to optimally segregate ecological effects of environmental variables and biotic interactions from the individual to the ecosystem level.

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CHAPTER 4:
**CLIMATE-TREE GROWTH ANALYSIS AT THE DROUGHT-
LINKED DISTRIBUTION EDGE OF *PINUS SYLVESTRIS* AND
*P. NIGRA***

Asier Herrero, Andreas Rigling, Regino Zamora



ABSTRACT

Rear edge populations, those residing at the low-latitude margins of species ranges, represent a critical genetic diversity for species conservation, management and evolutionary potential. The study analyzes climate-radial growth relations for *Pinus sylvestris* and *P. nigra* at their drought-linked distribution limit in the Mediterranean basin. The study was carried out in native relict forest of the two species in SE Spain, analyzing radial growth variability and climate-growth relations over time and between different ecological conditions. The lack of strong precipitation signals found could reflect a high local adaptation to climate at the drought-linked distribution limit, according to their high genetic differentiation. However, widespread negative impact of previous September temperature may be a result of lengthen summer drought affecting carbon reserve storage. In contrast, high winter and spring temperatures boosted radial growth in most locations. Both negative and positive impacts of temperatures have gained relevancy in the last decades. Beside common patterns, site- and species-specific signals in climate-tree growth relationships were recorded. Tree-growth variability increases at medium and low elevations coupled to increasing aridity conditions but not at high elevations, indicating that low elevations would be more exposed to rapid climate changes. The balance between positive and negative effects of temperature in radial growth, and factors like local adaptation and site-specific ecological conditions, can determine future performance and persistence of *P. sylvestris* and *P. nigra* rear edge populations at its drought-linked distribution limit in Mediterranean basin, where great distributional changes are expected as a result of rapid climate changes.

Keywords: rear edge populations, dendroecology, climate-growth relations, tree growth variability, *Pinus sylvestris*, *Pinus nigra*.

INTRODUCTION

Climate change can alter plant species distribution through impacts in growth, survival and recruitment (Allen and Breshears 1998, Parmesan and Yohe 2003, Peñuelas et al. 2007). The dynamics of populations at species distribution margins are critical to determine and forecast climate-induced range shifts (Thomas et al. 2004, Hampe and Petit 2005). Populations residing at the low-latitude margin of species distribution ranges, defined as rear edge populations, represent a critical genetic diversity for species conservation, management and future evolutionary potential (Hampe and Petit 2005, Jump et al. 2009). Therefore, the understanding of rear edge population's performance becomes increasingly urgent.

Many tree species reach its southern distribution limit at the Mediterranean basin, a region especially vulnerable to climate change (Giorgi and Lionello 2008). Rear edge populations at Mediterranean areas are mainly drought-limited, as drought constraint its main demographic rates (Peñuelas et al. 2001, Castro et al. 2004a, Galiano et al. 2010, Linares and Tiscar 2010). However, biotic factors such as competition, browsing, pests and pathogens can exacerbate or diminish drought impacts (Hódar et al. 2003, Linares et al. 2009, Herrero et al. 2011). Many relic populations at species rear edge are located at mountain systems, where complex topography provides suitable climatic conditions for its persistence (Hampe and Petit 2005). Expected upward migrations of plant populations as the climate to which they are adapted displace, could convert mountains in dead-end streets for many rear edge populations. Recurrence of extreme droughts (Beniston et al. 2007, IPCC 2007, Briffa et al. 2009) and associated drought-induced growth declines and mortality events (Jump et al. 2006, Linares et al. 2009, Galiano et al. 2010), could lead to local extinctions at Mediterranean rear edge populations.

However, isolated relic populations at species rear edge often present high genetic differentiation and adaptation to local conditions (Hampe and Petit 2005), which could enhance tolerance to stressful dry conditions at distribution limit,

acting as stabilizing mechanism which promotes persistence (Lloret et al. 2011). Therefore, rear edge populations harbor considerable genetic diversity critical for evolutionary potential and conservation under increasing aridity conditions. Assessment of tree performance through dendroecological methods is considered a valuable tool to determine future persistence of rear edge populations (Jump et al. 2009, Eilmann and Rigling 2012).

The main objective of the present study is to analyze climate-tree growth relationships of *Pinus sylvestris* L. and *P. nigra* Arnold at rear edge populations in SE Spain. Both species are characterized by wide distribution areas, with the drought-linked limits in the southern Mediterranean basin. As climatic conditions are changing constantly, there is need to not only assess climate-tree growth relationships for a given time period, but also to assess changes over time, allowing us to discuss future shifts in rear edge populations' performance. These climate-tree growth analyses will be performed along ecological gradients. The combined analyses of climate-tree growth relations varying in space and time will provide insight of tree performance at the drought-linked limits of *P. sylvestris* and *P. nigra*.

Mediterranean *P. nigra* often occur at warmer and drier sites than boreo-alpine *P. sylvestris* (Blanco et al. 1997, Rouget et al. 2001). Therefore, we expect *P. nigra* radial growth to be less dependent on water availability and less impacted by high summer temperatures than *P. sylvestris* at high elevations, where both species coexist. For *P. sylvestris* we compare forests with south and north aspect. We hypothesize lower response to high summer temperatures but a higher response to precipitation on south aspect locations with usually warmer and drier conditions. Finally, we compare *P. nigra* growth responses to climate along an altitudinal gradient including sites at high (2000 m), medium (1700 m) and low elevations (1500 m). Accordingly, the highest response to rainfall and high summer temperatures is expected at low elevation sites. On the other side, warmer winters

are expected to have a stimulating effect at high elevations resulting in an earlier onset of the growing season (Richter and Eckstein 1990).

Tree growth variability was also measured for each species and location, in order to test if these rear edge populations follow the increasing trend recorded at regional scale in the Iberian Peninsula for *P. sylvestris* and *P. nigra* (Tardif et al. 2003, Andreu et al 2007, Martín-Benito et al. 2010). Summarizing, the present study analyze changes in climate-tree growth relationships and tree growth variability over time and between different ecological conditions at *P. sylvestris* and *P. nigra* rear edge populations. The today's' growth responses of these rear edge populations will be discussed in the context of expected future climate change. Specifically, differences in growth response will be tested 1) for *P. sylvestris* growing on north and south aspects; 2) for *P. nigra* growing along an altitudinal gradient; and 3) for *P. sylvestris* and *P. nigra* growing both at high elevation. Finally, changes in climate-tree growth relation and growth variability over time will be tested.

MATERIALS AND METHODS

STUDY SITE AND SAMPLING DESIGN

The study was conducted at Sierra de Baza Natural Park (SE Spain, 2°51'48''W, 37°22'57''N). Sierra de Baza, together with Sierra Nevada, represents the absolute southernmost distribution limit for *Pinus sylvestris* (Boratynski 1991). For *P. nigra*, Sierra de Baza is one of the southernmost populations in the Iberian Peninsula (Barbéro et al. 1998). The climate is Mediterranean, characterized by cold winters and hot summers, with pronounced summer drought (June-August). Figure 1 showed a climatic diagram for period 1920-2007 using data from CRU

T.S 2.1 database (see Climatic data subsection), with a mean annual rainfall of 482 ± 12 mm (mean \pm SE). The bedrock is predominantly calcareous.

The study was performed on *P. sylvestris* subsp. *nevadensis* (Christ) and *P. nigra* relict native forests. To test the influence of aspect on *P. sylvestris* radial growth two different sites were sampled on 2000 m a.s.l., with south and north aspect, being only 300 m apart from each other in the same valley. While the forest on north aspect is formed exclusively by *P. sylvestris*, the forest on south aspect is composed by both *P. sylvestris* (56.08 ± 5.21 %) and *P. nigra* (43.92 ± 5.21 %), allowing growth responses of the two species to be compared. In addition, two *P. nigra* sites were sampled at 1700 (almost pure *P. nigra*) and 1500 m (63.41 ± 5.12 % *P. nigra*, 36.59 ± 5.12 % *Quercus ilex*) to analyze *P. nigra* growth responses along an altitudinal gradient. Characteristics and location of sampled sites are described in Table 1. At each site 20 trees were cored with an increment borer at breast height (1.30 m). Two cores were taken from each tree in two radial opposite directions. Diameter at breast height and tree height were recorded for each tree. Tree height was measured with Vertex IV hypsometer (Haglöf, Sweden).

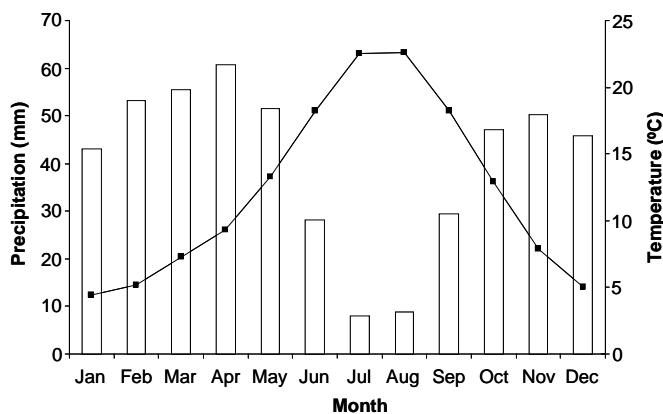


Figure 1 Climatic diagram for period 1920-2007 at the study area. Bars indicate monthly total precipitation and line monthly mean temperature.

Table 1 Characteristics of sampled sites and trees, and reliable time span. Elevation, aspect, geographical location and tree density are related to whole sites. Age at coring height, tree height and DBH are related to sampled trees. Finally, reliable time span for each chronology is shown. Note that *P. sylvestris* and *P. nigra* sites with south aspect are the same (mixed forest of both species)

Species	Elevation (m)	Aspect	Latitude (N)	Longitude (W)	Tree density (ind/ha) ^a	N° of trees	Age at coring height (yr)	Tree height (m)	DBH (cm)	Reliable time span ^b
<i>P. sylvestris</i>	2000	N	37° 22'	2° 51'	365 ± 29.08	17	104 ± 5.57	8.88 ± 0.46	45.22 ± 1.99	1908-2007
<i>P. sylvestris</i>	2000	S	37° 22'	2° 51'	252 ± 30.52	13	115.54 ± 6.57	8.68 ± 0.46	47.62 ± 3.61	1879-2007
<i>P. nigra</i>	2000	S	37° 22'	2° 51'	252 ± 30.52	15	139.6 ± 8.92	9.66 ± 0.51	49.70 ± 1.77	1867-2007
<i>P. nigra</i>	1700	NW	37° 24'	2° 49'	461 ± 39.80	11	97.72 ± 8.25	9.58 ± 0.40	36.72 ± 1.93	1935-2007
<i>P. nigra</i>	1500	NW	37° 24'	2° 50'	471 ± 46.64	12	104.17 ± 6.83	8.88 ± 0.34	34.79 ± 1.23	1916-2007

^a Tree density was measured with 20 transects of 50 m length and 10 m wide

^b EPS > 0.85

Table 2 Summary statistics for *P. sylvestris* and *P. nigra* chronologies for period 1935-2007: n° of trees, mean radial growth, mean sensitivity (MS), standard deviation (SD), first-order autocorrelation (r1), signal-to-noise ratio (SNR) and variance explained by the first principal component (VARpc1)

Species	Elevation (m)	Aspect	N° of trees (radii)	Radial growth mean (SD) (mm)	Standard chronology			Residual chronology		Detrended series: common period analysis	
					MS	SD	r1	MS	SD	SNR	VARpc1 (%)
<i>P. sylvestris</i>	2000	N	17 (34)	1.74 (0.72)	0.15	0.17	0.51	0.17	0.15	17.87	40.0
<i>P. sylvestris</i>	2000	S	13 (26)	1.30 (0.62)	0.19	0.24	0.66	0.18	0.18	25.78	52.1
<i>P. nigra</i>	2000	S	15 (30)	1.11 (0.42)	0.15	0.20	0.49	0.17	0.17	22.62	46.7
<i>P. nigra</i>	1700	NW	11 (22)	1.53 (0.88)	0.30	0.28	0.42	0.27	0.23	6.31	35.2
<i>P. nigra</i>	1500	NW	12 (24)	1.29 (0.84)	0.37	0.33	0.38	0.35	0.28	12.11	43.3

SAMPLE PROCESSING AND CHRONOLOGY BUILDING

Cores were mounted and sanded with progressively finer grades of sandpaper to highlight ring-width patterns. All cores were visually cross-dated following the procedures described by Yamaguchi (1991). Afterwards, tree-ring width was measured using a combination of a Lintab digital positioning table and the Time Series Analysis Program (TSAP, Rinntech, Germany). Because of numerous missing rings, cross-dating was not always possible and the corresponding cores were excluded. The final number of trees used and tree characteristics are shown in Table 1.

Each ring-width series was detrended with a 30-year cubic smoothing spline with a 50 % frequency response (Cook and Peters 1981) using the software ARSTAN (Holmes 1994). Each measured series was standardized dividing observed values by predicted values to obtain dimensionless ring-width indices series. By this way, climatic-signal was amplified and the effect of non-climatic factors such as age-related trends were removed (Fritts 1976). Index series were averaged using a bi-weight robust mean, which reduces the variance and bias caused by extreme values, to develop the mean standard chronology. Additionally, temporal autocorrelation was removed applying autoregressive modelling and the residual chronology developed. This procedure was performed for each sampled site and species.

The reliable time span was calculated for each chronology, based on a minimum expressed population signal (EPS) value of 0.85, which is widely used as a threshold value in dendroclimatic studies (Wigley et al. 1984). EPS quantifies the degree to which a particular sample chronology depicts a hypothetically perfect chronology (Briffa and Jones 1990). The reliable time span includes at least cores from five trees in all the cases. Several descriptive statistics were calculated for period 1935-2007 for all chronologies, as this period was common for all the chronologies (see Table 1). Mean radial growth, mean sensitivity (MS), standard

deviation (SD), first-order autocorrelation (r_1), signal-to-noise ratio (SNR) and variance explained by the first principal component (VARpc1) were calculated. MS, SD and r_1 are used to assess statistically the dendroclimatological quality of the tree-ring chronologies. MS measures the relative differences in the widths of adjacent growth rings, and r_1 describes the influence of the previous growth on the growth of the current year (Fritts 1976). While MS and r_1 measure high- and low-frequency variation in radial growth respectively, SD measures variation in both frequencies. SNR and VARpc1 were calculated for detrended series. SNR measures the common variance in a chronology scaled by a measure of the total variance of the chronology (Wigley et al. 1984, Cook et al. 1990). Finally, the VARpc1 is a measure of the strength of the common growth signal.

TREE-GROWTH VARIABILITY

To analyze tree-growth variability between different species and sites, years with extreme growth values, defined as those ring-width indices that exceed 1.75 standard deviations from the mean of the residual chronology, were identified for the period 1935-2007. In addition, annual sensitivity (s_x) was calculated by dividing the absolute value of the differences between each pair of ring-width indices by the mean of the paired index (Fritts 1976). To assess trends in s_x , mean annual sensitivity (ms_x) was computed for 38-year periods and shifted by intervals of 5 years. Trends in growing season (May-October) mean temperature and total precipitation for the same intervals were also considered.

CLIMATIC DATA

The climatic data used were monthly mean temperature and total precipitation for the study area extracted from CRU TS 2.1 high-resolution gridded data set (Mitchell and Jones 2005). As this set encompassed only the 1901-2002 period, we performed linear regressions using data from nearby meteorological stations to extend monthly data to 2007 (all the regressions were significant at $P < 0.05$). To test climate trends for the period 1920-2007, linear regressions were performed between monthly and annual mean values and time (years). We selected this period to correspond to the same interval used in moving response function analysis (see below).

GROWTH-CLIMATE RELATIONSHIPS

To quantify growth-climate relationships between residual chronologies of each species and site and climate series (monthly mean temperature and precipitation sums), correlation and response function analyses were performed using the software Dendroclim2002 (Biondi and Waikul 2004). Correlation analysis corresponds to Pearson's product moment correlation. Response function is a principal component regression which accounts for multicollinearity in multivariable sets of climatic data (Fritts 1976). Significance of correlation and response function coefficients were tested at 0.05 level using 1000 bootstrapped estimates, drawn at random with replacement from the initial data set (Biondi and Waikul 2004). Growth-climate relationships were analyzed from the previous June to October of the growth year for the common period 1935-2007. In addition, changes in radial growth response to climate through time were assessed using moving response function analysis with 68-year fixed intervals, increasing both start and end year by one for each iteration. This analysis was performed for the

period 1920-2007 to achieve necessary number of degrees of freedom, excluding *P. nigra* chronology at 1700 m elevation. For changes in growth-climate relationships, we focused on response function analysis rather than correlation, as the former is more conservative. Nevertheless, the observed changes in response function coefficients are in agreement with changes in correlation coefficients.

RESULTS

CHRONOLOGY STATISTICS

P. sylvestris mean radial growth was higher on north aspect than on south aspect (Table 2). For *P. nigra*, the highest mean radial growth was found at medium elevation (1700 m), following by low (1500 m) and high elevation (2000 m). At high elevation south aspect, *P. sylvestris* displayed higher mean growth than *P. nigra*. MS in *P. sylvestris* was higher on south aspect than on north aspect for both standard and residual chronologies (Table 2). For *P. nigra*, the highest values of MS were found at the lowest site and the values decreased with altitude (similar results for standard and residual chronologies; Table 2). SD showed the same pattern recorded for MS. r_1 values were higher for *P. sylvestris* than for *P. nigra*, with the highest values recorded for *P. sylvestris* on south aspect and the lowest for low elevation *P. nigra* (Table 2). SNR ranged from 6.31 (medium elevation *P. nigra*) to 25.78 (south aspect *P. sylvestris*), and VARpc1 from 35.2 (medium elevation *P. nigra*) to 52.1 (south aspect *P. sylvestris*; Table 2). Residual chronologies and associated core numbers are plotted in Figure 2.

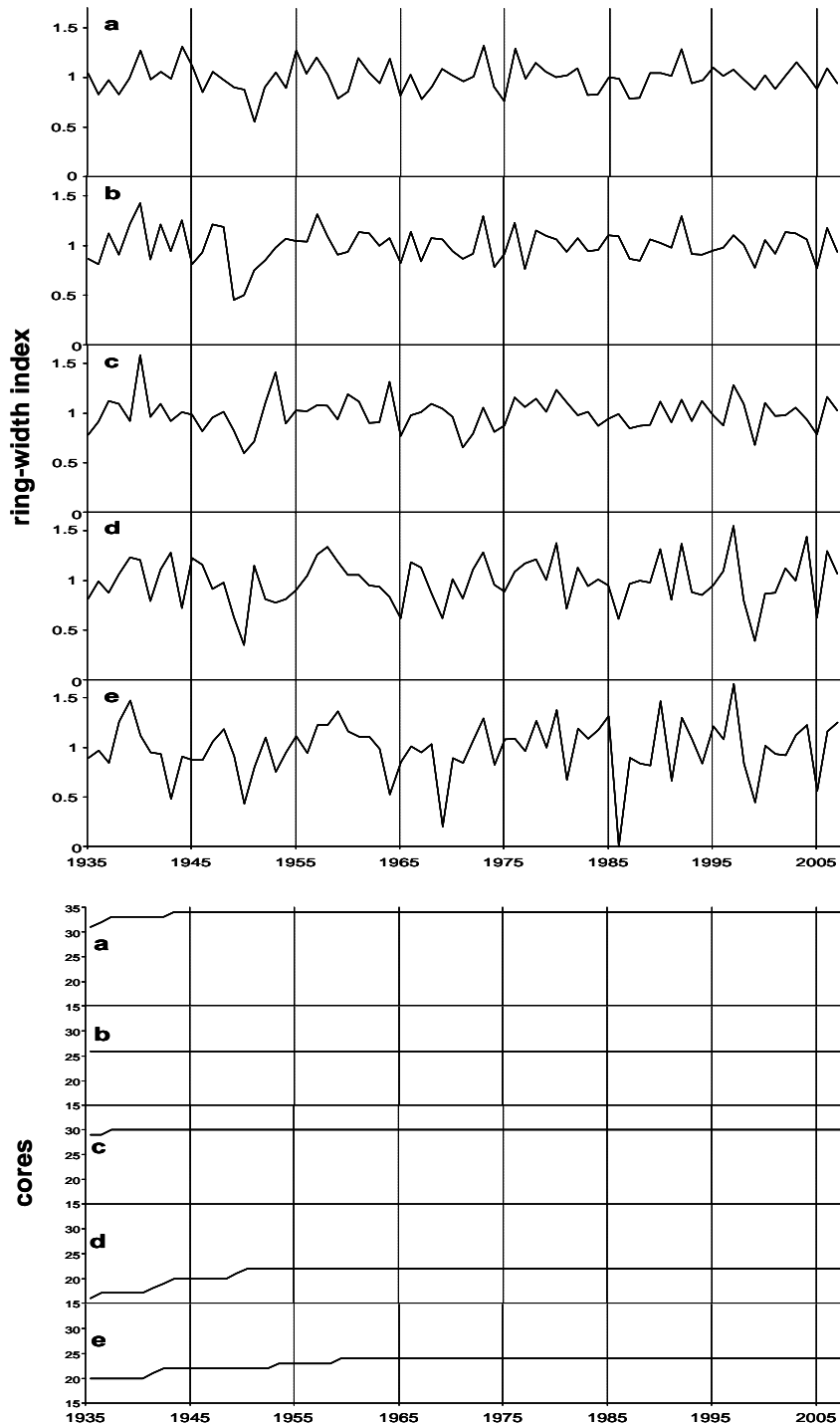


Figure 2 Residual chronologies of *P. sylvestris* and *P. nigra* for period 1935-2007 and the corresponding number of cores. For *P. sylvestris*, chronologies of north (a) and south aspect (b) at 2000 m elevation are shown. For *P. nigra*, chronologies at 2000 (a), 1700 (b), and 1500 m of elevation (c) are shown.

TREE-GROWTH VARIABILITY

Frequency of narrow (< 1.75 SD) and wide (> 1.75 SD) ring-width indices displayed differences between species and locations (Fig. 3). *P. nigra* at medium and low elevations presented the highest frequency of extreme values, showing both an increasing trend in the last decades. *P. sylvestris* on south aspect showed higher frequency of extreme narrow ring-width indices for the period 1935-1955 than coexisting *P. nigra* and *P. sylvestris* on north aspect (Fig. 3). ms_x also showed an increasing trend at medium and low elevations during the past decades, coupled with increasing aridity (warmer and drier conditions) in the growing season (Fig. 4). However, *P. sylvestris* and *P. nigra* at high elevations did not show any increasing trend in ms_x .

CLIMATIC DATA

Annual mean temperature and the monthly temperatures in March and especially in June and July significantly increased (Fig. 5). Total precipitation decreased significantly in March, and increased slightly in May. Overall, annual precipitation showed a significant negative trend (Fig. 5).

GROWTH-CLIMATE RELATIONSHIP

The negative effect of previous (t-1) September temperature was a strong common signal for both species and all the study sites, significant for both correlation and response function analyses (Fig. 6). Temperature signals prevailed over precipitation, in fact, only current (t) May precipitation showed both significant positive correlation and response function coefficients for *P. nigra* at

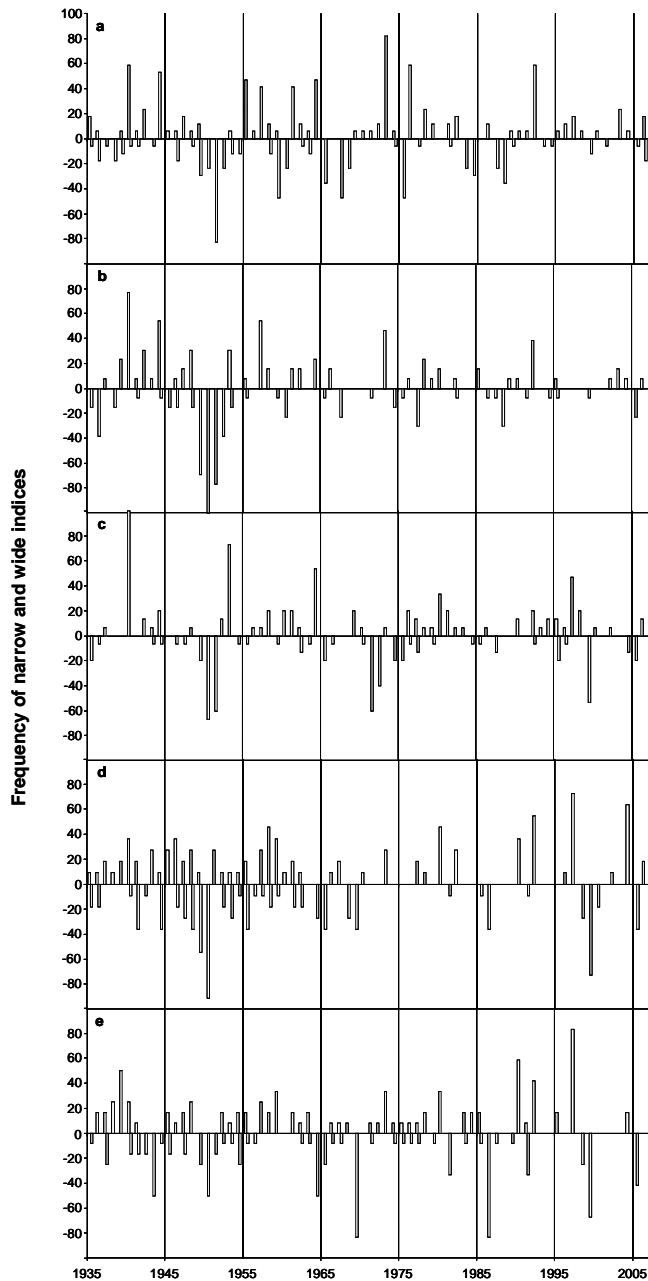
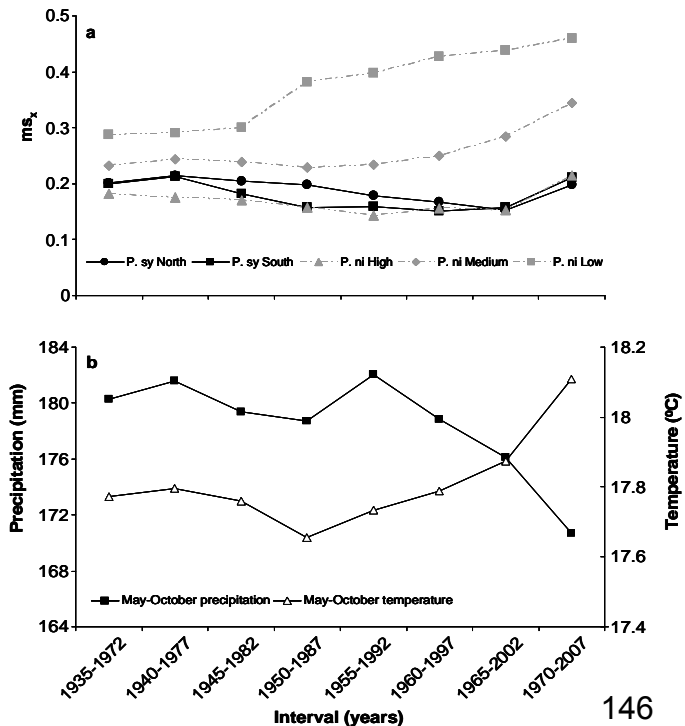


Figure 3 Relative frequency of narrow (< 1.75 SD) and wide (> 1.75 SD) ring-width residual chronology indices for each species and location during the period 1935-2007: north (a) and south aspect (b) *P. sylvestris*, and *P. nigra* at 2000 (c), 1700 (d) and 1500 m of elevation (e).

low elevation. Other significant positive signals in precipitation were recorded only for correlation analysis: June (t) at high elevation for the two species, October (t-1) for *P. nigra* at high and medium elevation, and October (t) for *P. sylvestris* on south aspect. Finally, September (t) precipitation showed a significant negative correlation with radial growth for *P. nigra* at high elevation (Fig. 6).

Temperature showed stronger signals, often significant for both correlation and response function analysis (Fig. 6). Positive strong signals detected by both analyses are concentrated in the winter and spring months: May (t) for *P. sylvestris* north aspect, March (t) for *P. nigra* at high elevation, February (t) for *P. nigra* at medium and low elevation, and December (t) for *P. nigra* at low elevation. Apart from the mentioned September (t-1) signal, only August (t-1) temperature for *P. nigra* at high elevation displayed significant negative correlation and response function coefficients. Other negative relationships between monthly temperatures and growth were significant only for correlation analysis: July (t) for *P. nigra* at all elevations, July (t-1) for *P. nigra* at high elevation, and August (t-1) for *P. nigra* at medium elevation. In addition, May (t) temperature displayed a significant positive response function coefficient for *P. nigra* at high elevation (Fig. 6).

Figure 4 Temporal trends in mean annual sensitivity (ms_x) and mean growth season temperature and total precipitation. Intervals of 38 years lagged 5 years are used. Growing season includes months from May to October. P. sy: *Pinus sylvestris*; P. ni: *Pinus nigra*.



GROWTH-CLIMATE RELATIONSHIP THROUGH TIME

Figure 7 shows the temporal evolution of response function coefficients of variables that were significant for both correlation and response function analyses per species and site (with the exception of *P. nigra* at medium elevation). There was a general increase over time of the common negative effect of September (t-1) temperature. For *P. nigra* at high elevation, there was also an increase of the negative effect of August (t-1) temperature, becoming significant in the last considered intervals. In contrast, there was an increase of the positive effects of winter and spring temperatures for *P. sylvestris* north aspect (May t), and for *P. nigra* at high (March t) and low elevations (December t-1, February t). May (t) precipitation also increased its response function coefficients in the last intervals for *P. nigra* at low elevation.

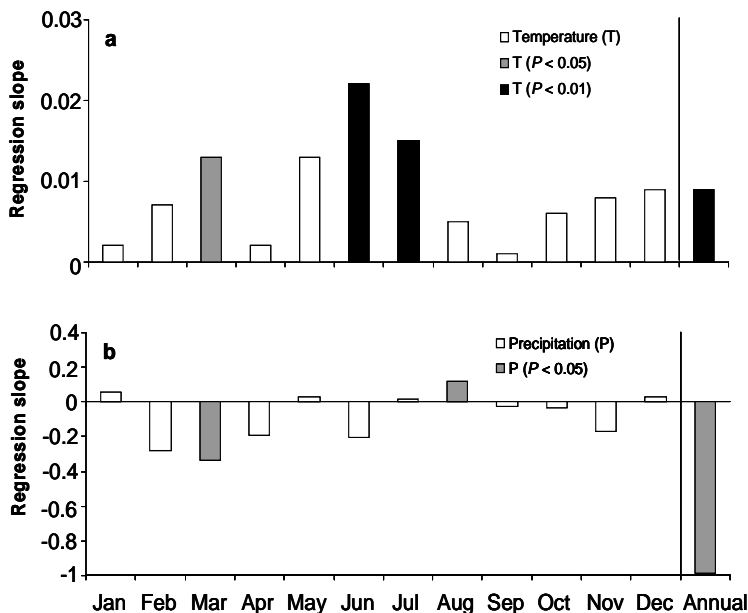


Figure 5 Trends (slope of the linear regression) in monthly and annual mean temperature (a) and total precipitation (b) for period 1920-2007. Grey bars indicate significant linear regression at $P < 0.05$, and black bars at $P < 0.01$.

DISCUSSION

Our climate-tree growth analysis revealed prevailing temperature signals for *P. sylvestris* and *P. nigra* rear edge populations. The negative effect of September (t-1) temperature was common to all locations, recording also a widespread positive effect of winter or spring temperatures. Both negative and positive impacts of temperatures have gained relevancy in the last decades. Despite recorded common patterns, site- and species-specific climate-tree growth relationships were recorded, as well as differences in tree-growth variability. *P. nigra* at medium and low elevations showed the highest growth variability, displaying also an increasing trend over time coupled with increasing drier conditions.

TREE-RING CHRONOLOGIES

Lower growth and higher MS for *P. sylvestris* at south than at north aspect suggest more stressful conditions, as MS is expected to increase with increasing drought stress (Fritts et al. 1965, Rigling et al. 2001, 2003). However, r1 was higher at south aspect, contrary to recorded pattern between dry and mesic sites for *P. sylvestris* at inner Alpine dry valleys (Rigling et al. 2002). VARpc1 was also higher in south aspect, although more limiting environmental conditions could increase similarities in climate response between individuals (Eilmann et al. 2009). For *P. nigra*, the highest MS and the lowest r1 values at low elevation point out this location as the most stressful. This is in accordance to other studies performed with southern *P. nigra* populations, which recorded drought-induced growth declines at low elevations (Linares and Tíscar 2010, 2011).

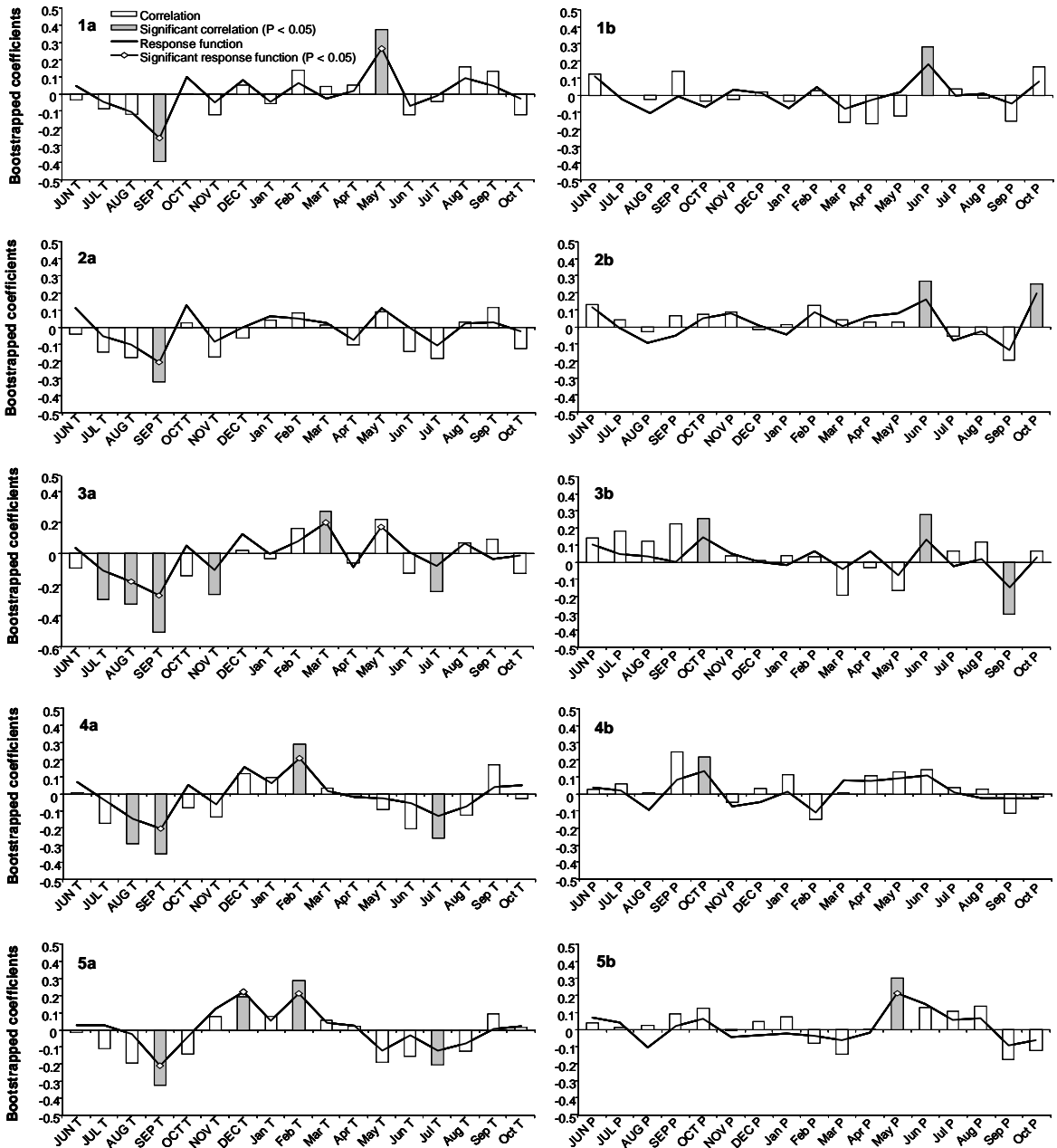


Figure 6 Bootstrapped correlations (bars) and response functions (lines) performed between residual chronologies and monthly temperature (a) and precipitation (b) data from prior June to current October. Prior and current year months are indicated by capitals and lowercase, respectively. Data for north (1) and south aspect (2) *P. sylvestris* chronologies, and 2000 (3), 1700 (4), and 1500 m of elevation (5) *P. nigra* chronologies are shown. Significant correlations and response function coefficients ($P < 0.05$) are indicated by gray bars and white diamonds, respectively.

TREE-GROWTH VARIABILITY

Recent studies recorded an increase of frequency of extreme growth values at regional scale for several conifers in the second half of 20th century, coupled to an increased variability in climate and increased aridity conditions (Tardif et al. 2003, Andreu et al. 2007). In fact, high frequency of extreme growth values indicates periods of high climatic influence (Tardif et al. 2003). In the present study, an increase of extreme growth values in the last decades was detected for *P. nigra* at medium and low elevations, but not at high elevation. This indicates that low elevations would be more exposed to recorded climate changes than high elevations. In fact, more severe drought-induced growth reductions and mortality events were recorded at low than at high elevations (Allen and Breshears 1998, Adams and Kolb 2004, Linares and Tiscar 2011, Dobbertin et al. 2005). However, some differences in tree growth variability were also recorded at high elevations. For the period 1945-1955, *P. sylvestris* at south aspect showed a higher frequency of narrow extreme values than *P. sylvestris* at north aspect and coexisting *P. nigra*. This result suggests a higher vulnerability to drought for *P. sylvestris* at south aspect, as the considered period was characterized by recurrent severe droughts (data not shown).

ms_x increased in the last decades for *P. nigra* at medium and low elevations coupled with decreasing precipitation and increasing temperature trends, showing a similar pattern to the frequency of extreme growth values. The absence of increasing trends in ms_x at high elevations could be due to less limiting climatic conditions at these cooler and more moist sites. Therefore, our results prove that recorded general increases in tree growth variability at regional scale (Tardif et al. 2003, Andreu et al 2007, Martín-Benito et al. 2010), can vary at local scale linked to site-specific ecological conditions.

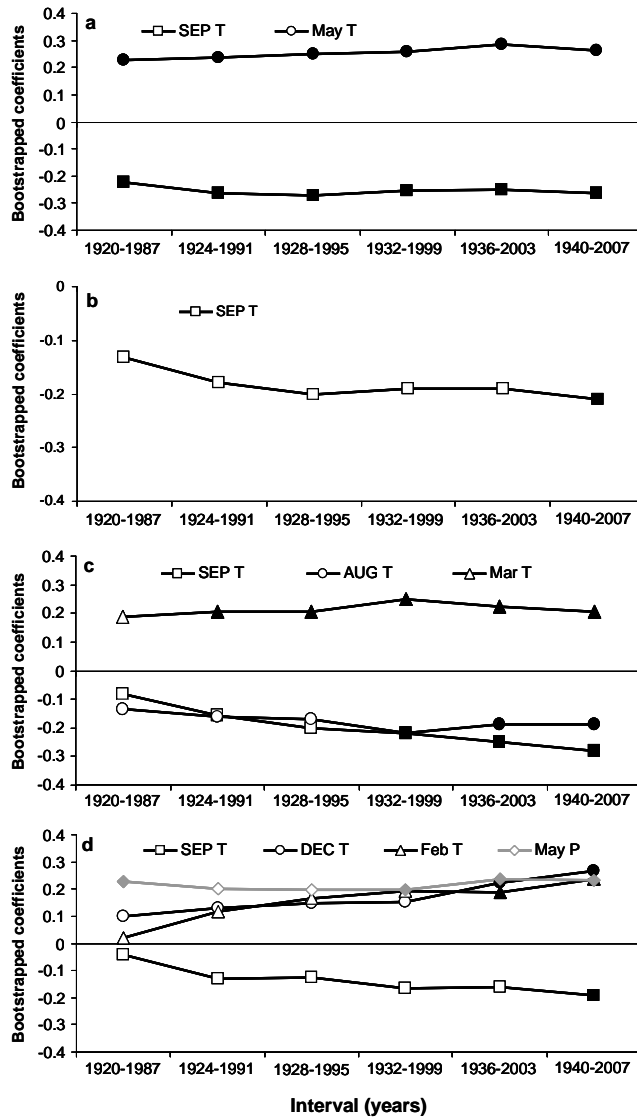


Figure 7 Temporal changes in bootstrapped response function coefficients using intervals of 68 years lagged 4 years for north aspect (a) and south aspect (b) *P. sylvestris* chronologies, and *P. nigra* chronologies at 2000 (c) and 1500 m of elevation (d). Only variables significant for both common period correlation and response function analysis are shown for each chronology. Prior and current year months are indicated by capitals and lowercase, respectively. Filled symbols indicate significant response function coefficients ($P < 0.05$).

GROWTH-CLIMATE RELATIONSHIP

The lack of strong precipitation signals, with the exception of *P. nigra* at low elevation, is unexpected at the southernmost distribution limit of *P. sylvestris* and *P. nigra*. At this latitude, establishment, growth and survival of both species are constrained by water availability (Martínez-Vilalta and Piñol 2002, Castro et al. 2004a, 2004b, Linares and Tíscar 2010, 2011). Thus, we expected more strong positive precipitation signals, even more if we consider the negative trend in precipitation recorded in the study area. In fact, recorded results contrast with other studies performed in southern populations of Spain and other drought-limited areas of e.g. the European Alps showing dominant influence of precipitation (Martín-Benito et al. 2010, Weber et al. 2007, Eilmann et al. 2009, Affolter et al. 2010). However, similar lack of precipitation signals has been recorded for *P. sylvestris* from northern Spain (Andreu et al. 2008). The absence of strong precipitation signals could reflect a high local adaptation to climate at species drought-linked distribution limits. In fact, southern populations of *P. nigra* and *P. sylvestris* in Spain have been isolated from northern populations for long periods, and present high level of genetic differentiation (Prus-Glowacki and Stephan 1994, Afzal-Rafii and Dodd 2007). For instance, *P. sylvestris* subsp. *nevadensis* presented in the study area a lower vulnerability to embolism than other northern populations (Martínez-Vilalta et al. 2009). In addition, heterogeneous topography at mountain systems could ameliorate the negative impact of drought through microclimate conditions (Hampe and Petit 2005) such fog persistence (personal observation).

However, high temperatures can exacerbate drought stress for trees (Adams et al. 2009). The observed widespread negative impact of September (t-1) temperature has been previously reported for *P. nigra* and *P. sylvestris* (Andreu et al. 2007, Martín-Benito et al. 2010, Rigling et al. 2002), and may be a result of lengthen summer drought. An increased drought stress in late summer can impact storage of carbon reserves and negatively affect radial growth in the next year

(Fritts 1976, Rolland and Schueller 1994). In contrast, high winter and spring temperatures boosted radial growth in most locations. On the one hand, May (t) temperature positively influenced *P. sylvestris* radial growth at north aspect, probably as a result of earlier onset of growing season (Richter and Eckstein 1990). On the other hand, higher winter temperatures might prevent the more thermophilic *P. nigra* from frost damages with a positive effect on radial growth. The absence of strong precipitation signals makes the balance between positive and negative effects of temperature decisive for *P. sylvestris* and *P. nigra* performance at its southernmost distribution limit.

Beside common patterns, site- and species-specific signals in climate-tree growth relationships were recorded. At high altitude, *P. sylvestris* showed a strong positive temperature signal in May (t) but only on north and not on south aspect. Hence in this cool location a warm May is beneficial for initiating tree growth. *P. nigra* showed some differences along the elevation gradient. May (t) precipitation positively affected radial growth at low elevation, being the only strong precipitation signal. This indicates higher sensitivity to hydric stress at low elevation, as has been reported in several studies (Adams and Kolb 2004, Linares and Tiscar 2010, 2011, Affolter et al. 2010). Finally, *P. sylvestris* and *P. nigra* showed striking differences in climate-tree growth relationships at high elevation on south aspect. Summer temperatures of the previous and the current year exerted higher negative influence in Mediterranean *P. nigra* than in coexisting boreo-alpine *P. sylvestris*. We expected the opposite pattern inferred by their biogeographical origin and the more thermophilic nature of *P. nigra* (Barbéro et al. 1998, Rouget et al. 2001). However, this negative impact of warm temperatures could be balanced by higher positive influences of winter and spring temperatures for *P. nigra*.

GROWTH-CLIMATE RELATIONSHIP THROUGH TIME

The widespread negative effect of September (t-1) temperature increased in the last decades in all locations, probably as a result of increasing temperatures. Surprisingly, no specific trend was reported neither for September temperature nor precipitation. However, September is a key month when normally the drought period ends and even little increase in temperature could aggravate drought stress. This might be amplified by the overall decrease of precipitation in the Mediterranean during the past decades, resulting in generally drier conditions (Carnicer et al. 2010). Positive impacts of spring and winter temperatures also increased in the last decades, according to recorded increasing trends in temperature in the study area. Thus, the future performance of these rear edge populations will depend in the final outcome of negative and positive influences of temperatures, which showed an overall increasing trend.

CONCLUSIONS

The balance between positive and negative effects of temperature in radial growth, and factors like local adaptation and site-specific ecological conditions, can determine future performance and persistence of *P. sylvestris* and *P. nigra* rear edge populations at its drought-linked distribution limit in Mediterranean basin, where great distributional changes are expected as a result of rapid climate changes. Thus, it seems necessary to include both site-specific conditions and local adaptation knowledge in theoretical models to improve the predictions about species distributional shifts and local extinctions. This information can also be used to improve management practices and conservation strategies of rear edge populations, which harbor considerable and valuable genetic diversity.

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CHAPTER 5:
PLANT RESPONSES TO EXTREME CLIMATIC EVENTS: A
FIELD TEST OF RESILIENCE CAPACITY AT THE
SOUTHERN RANGE

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ABSTRACT

The expected and already observed increment in frequency of extreme climatic events can result in severe vegetation shifts. However, stabilizing mechanisms promoting community resilience can buffer the lasting impact of extreme events. The present work analyzes the resilience of a Mediterranean mountain ecosystem to an extreme drought in 2005, examining shoot-growth and needle-length resistance and resilience of dominant tree and shrub species situated at their southernmost distribution limit: *Pinus sylvestris* and *Juniperus communis* along the oromediterranean belt, and *P. nigra* and *J. oxycedrus* in the montane belt. Resistance and resilience components vary across species, sites, and ontogenetic states: adult *Pinus* showed higher growth resistance than did adult *Juniperus*; saplings displayed higher recovery rates than did conspecific adults; and *P. nigra* displayed higher relative growth resilience than did *P. sylvestris* where the two species coexist. *P. nigra* and *J. oxycedrus* saplings at high and low elevations, respectively, were the most resilient at all the locations studied. Under a scenario of recurrent extreme droughts, observed resilience differences coupled with adult species-specific drought vulnerability could favor a change in dominance towards these species at each altitudinal belt. However, recorded growth-resilience values were overall high, indicating great tolerance to extreme droughts for the dominant species of pine-juniper woodlands. Observed tolerance could act as a stabilizing mechanism in rear range edges, such as the Mediterranean basin, where extreme events are predicted to be more detrimental and recurrent.

Keywords: resistance, resilience, extreme drought, Mediterranean mountain, pine-juniper woodland.

INTRODUCTION

The expected and already observed increment in frequency and severity of extreme climatic events (Della-Marta et al. 2007, IPCC 2007, Briffa et al. 2009) urgently requires knowledge about ecosystem resistance and resilience. Resistance can be considered as an ecosystem, community or individual opposing force to change exerted by an external disturbance (MacGillivray et al. 1995), and resilience the capacity to restore pre-disturbance structure and function (analogous to ‘engineering resilience’, see Holling 1996). Recurrent extreme events could disrupt ecosystem processes and structures of a particular state, and induce a catastrophic shift with associated loss of essential ecosystem services (Scheffer et al. 2001, Folke et al. 2004). The study of ecosystem responses in terms of resistance and resilience to extreme events can help to forecast ecosystem changes, as future average conditions will be close to current extreme events (Battisti and Naylor 2009).

Extreme drought and warm events are closely related to growth reduction and mortality of woody species in forest ecosystems across the planet (Allen et al 2010). Recurrent and extreme droughts impact woody species performance differently through species-specific sensitivity, leading to changes in species composition (Allen and Breshears 1998, Slik 2004, Mueller et al. 2005, Bigler et al. 2006, Koepke et al. 2010, Zweifel et al. 2009). Furthermore, differences in drought sensitivity between functional types, such trees and shrubs, can alter vegetation structure, shifting from a tree-dominated landscape to shrub-dominated one (Allen and Breshears 1998, Mueller et al. 2005, Koepke et al. 2010). However, stabilizing processes promoting community resilience can palliate and offset the aftermath of extreme events (Lloret et al. 2011a). While phenotypic plasticity can buffer rapid climate changes and assist adaptation (Nicotra et al. 2011), contrasting environmental conditions can provide adequate sites for survival and performance in heterogeneous landscapes (Suarez et al. 2004, Lloret et al. 2004). Therefore, the

analysis of woody species resistance and resilience is particularly crucial under the rising frequency of extreme events and associated forest decline.

In addition to the comparison of responses to extreme events between species and functional types, it is necessary to compare ontogenetic states. Environmental responses at the individual level can vary greatly with age, due to increases in size and structural complexity (Szeicz et al. 1994, He et al. 2005). Size-related changes include increases in the volume and depth of soil explored by the root system, which provides access to more stable soil moisture and nutrient supply at deeper soil layers (He et al. 2005, Thomas and Winner 2002). Greater height imply greater hydraulic resistance against the transport of water, photosynthate, and hormone molecules over longer distances (He et al. 2005, Thomas and Winner 2002), leading to different sensitivity to extreme events between saplings and adults. Therefore, due to the importance of ontogenetic changes and the fundamental role of saplings in forest regeneration and expansion, comparison of resistance and resilience between adults and saplings will provide a wide perspective of forest responses to extreme events.

The main objective of this study is to analyze the resistance and resilience of a Mediterranean mountain ecosystem to an extreme drought event in 2005, monitoring performance of dominant forest species before, during, and afterwards. Boreo-alpine *Pinus sylvestris* L. subsp. *nevadensis* Christ and *Juniperus communis* L. are the dominant species along the oromediterranean belt (1800-2000 m a.sl.), while Mediterranean *Pinus nigra* Arnold and *Juniperus oxycedrus* Sibth & Sm are the dominant ones in the montane belt (1400-1700 m). All the species showed one flush per year in the study area and bore multiple needle cohorts in the same branch, enabling shoot and needle growth changes to be compared. The monitored species were situated at their southernmost distribution limit, forming natural relict populations in the study area (Blanco et al. 1997). Although populations at the species rear edges are considered more vulnerable to extreme events and climate-change impacts, past persistence observed in many relict populations (Hampe and

Petit 2005) suggest high tolerance to extreme climatic events. We hypothesize that monitored species living at the edge of their southern range will present high resistance and resilience to extreme drought events due to high genetic differentiation and local adaptation commonly found at species rear edges (Hampe and Petit 2005). Thus, the analysis of multi-species resilience at rear edges as performed in this study will help to forecast species distributional shifts under a changing climate scenario.

With respect to comparing functional types, we expect lower resistance and resilience in trees than in shrubs, due to differences in hydraulic architecture. Isohydric *Pinus*, in contrast to anisohydric *Juniperus*, prevent hydraulic failure during drought conditions by severely reducing stomatal conductance (McDowell et al. 2008, Zweifel et al. 2009). However, this lower stomatal conductance depresses photosynthetic carbon uptake that could limit pine resistance and ensuing recovery. For each species, large mature adults and non-reproductive saplings were also considered. Higher resilience of adults is expected as saplings possess shallower root systems and lower carbon reserves. However, lower resilience of adults is also possible due to higher vulnerability to xylem embolism, greater water use per unit of time (Slik 2004) and slower shoot growth rates (Day and Greenwood 2011).

In addition, the two pine species are compared in locations where they coexist. A priori, lower resilience to an extreme drought can be inferred for *P. sylvestris* from its biogeographical origin. Apart from species comparisons, pine species resistance and resilience to 2005 extreme drought is compared under different ecological conditions. The populations located at higher elevations and/or northern exposures are expected to show higher resilience than populations at lower elevations and/or southern exposures due to wetter and cooler conditions in the former.

In summary, these are the specific questions addressed in the present study:

1) Are tree species showing lower resilience than shrubs? 2) Are adults showing

lower resilience than saplings? 3) Is *P. sylvestris* showing lower resilience than *P. nigra* where both coexist? 4) Are Pine species showing lower resilience at low elevation and/or southern exposition?

MATERIAL AND METHODS

STUDY SITE AND SPECIES

The study was conducted at Sierra de Baza Natural Park (SE Spain, 2°51'48''W, 37°22'57''N). The climate is Mediterranean, characterized by cold winters and hot summers, with pronounced summer drought (June-August). Precipitation is concentrated mainly in autumn and spring. The annual and summer rainfall is 495 ± 33 mm and 31 ± 9 , respectively (mean \pm SE for period 1991-2006; Cortijo Narváez meteorological station, 1360 m a.s.l.). The bedrock is predominantly calcareous. The study species are the dominant ones in their altitudinal belt, forming characteristic vegetation types. In the oromediterranean belt (1800-2000 m a.s.l.), while *P. sylvestris* subsp. *nevadensis* is the main tree species, *J. communis* is the main shrub covering the forest understory and open areas. On the other hand, in the montane belt (1400-1700), *P. nigra* and *J. oxycedrus* are the dominant species. In 2005 the most extreme drought in the last six decades occurred in Western Europe (Garcia-Herrera et al. 2007). In fact, climatic records from the study area (Cortijo Narváez meteorological station) revealed that 2005 was the driest year since 1947.

SAMPLING DESIGN

Different *P. sylvestris* and *P. nigra* populations were monitored in natural relict forests at Sierra de Baza. *P. sylvestris* populations were sampled on north- and south-facing slopes of the same valley (2000 m), while *P. nigra* populations were monitored following an altitudinal gradient: at high (2000 m), medium (1700 m) and low elevations (1500 m). Only at 2000-m elevation in a south-facing forest, do *P. sylvestris* and *P. nigra* coexist to form a mixed forest. *J. communis* and *J. oxycedrus* were sampled at the same north-facing locations of *P. sylvestris* and low-elevation *P. nigra* populations, respectively. For each location, a plot of 1-2 ha surface was established, being at least 600 m away from each other. In each plot, large mature adults and non-reproductive saplings were sampled. See Table 1 for further information about monitored plots. All the measurements were made from 2005 to 2008.

Trees

For tree species, 10 representative mature trees and 15 saplings of similar size were recorded haphazardly in each plot. Individuals with significant herbivory or physical damages were avoided. Height and DBH (Diameter at Breast Height) in adults, and height, basal diameter, and age in saplings were recorded (see Table 1). Adult height was measured using a Vertex IV hypsometer (Haglöf, Sweden). Sapling age was estimated by counting the number of annual bud scars or whorls (Edenius et al. 1995). Longitudinal shoot growth in adults was measured in 10 branches per tree, five facing north and five south. Measured branches belonged to medium or low tree crown. In saplings, shoot growth was measured in the leader shoot. Shoot growth of each year was identified using annual whorls and yearly bud scars from 2003 to 2008. Needle length was measured in three needles per

Table 1 Adult and sapling size in each sampled plot. Cover area was calculated measuring major and minor canopy diameters. Values are shown as mean \pm standard error. DBH: Diameter at Breast Height.

Species	Altitude	Exposure	Plot	Adults			Saplings		
				Height (m)	DBH (cm)	Cover area (m ²)	Height (cm)	Basal diameter (cm)	Cover area (m ²)
<i>P. sylvestris</i>	2065	N	1	9.55 \pm 0.69	44.86 \pm 2.84	-	112.2 \pm 5.85	4.44 \pm 0.28	-
<i>P. sylvestris</i>	2037	N	2	7.64 \pm 0.26	43.35 \pm 2.82	-	93.33 \pm 8.12	3.68 \pm 0.33	-
<i>P. sylvestris</i>	2008	S	1	8.89 \pm 0.61	43.42 \pm 3.92	-	92.63 \pm 8.74	5.09 \pm 0.64	-
<i>P. sylvestris</i>	2067	S	2	7.78 \pm 0.36	49.07 \pm 3.75	-	110.93 \pm 8.33	4.07 \pm 0.43	-
<i>P. nigra</i>	2008	S	1	9.74 \pm 0.67	46.9 \pm 4.04	-	111.29 \pm 6.55	4.11 \pm 0.33	-
<i>P. nigra</i>	2067	S	2	9.79 \pm 0.62	49.31 \pm 2.80	-	99.47 \pm 7.51	4.39 \pm 0.33	-
<i>P. nigra</i>	1753	NE	1	9.6 \pm 0.35	34.10 \pm 1.20	-	101.63 \pm 6.73	3.3 \pm 0.17	-
<i>P. nigra</i>	1694	NW	2	8.74 \pm 0.52	35.86 \pm 2.51	-	103.87 \pm 6.10	4.52 \pm 0.25	-
<i>P. nigra</i>	1525	NW	1	8.51 \pm 0.6	31.06 \pm 2.06	-	99.61 \pm 6.18	4.88 \pm 0.28	-
<i>P. nigra</i>	1544	NE	2	8.69 \pm 0.24	33.78 \pm 1.03	-	92.63 \pm 5.9	4.2 \pm 0.17	-
<i>J. communis</i>	2048	NE	1	-	-	28.57 \pm 2.85	-	-	0.31 \pm 0.08
<i>J. communis</i>	2031	NW	2	-	-	14.44 \pm 1.18	-	-	0.11 \pm 0.02
<i>J. oxycedrus</i>	1525	NW	1	1.96 \pm 0.08	-	4.29 \pm 0.48	0.34 \pm 0.02	-	0.06 \pm 0.01
<i>J. oxycedrus</i>	1544	NE	2	1.9 \pm 0.11	-	4.52 \pm 0.49	0.44 \pm 0.03	-	0.14 \pm 0.02

shoot-growth cohort. In 2006 winter, 2003-2005 shoot cohorts were measured. In 2007 and 2008 late autumns, 2006-2007 and 2008 shoot cohorts were measured, respectively.

Shrubs

20 adults and 20 saplings of similar size were recorded haphazardly in each plot, avoiding individuals with significant herbivory or physical damages. With respect to adults, 10 males and 10 females were recorded in each plot. However, we pooled all adults due to the absence of differences between sexes in recorded variables. Height was recorded only for *J. oxycedrus*, as *J. communis* had a prostrate growth form in the study area. Basal diameter was not quantified due to difficulties to measure, particularly in prostrate *J. communis*, and to the common multi-trunk growth pattern. In return, major and minor canopy diameters were measured in all individuals, and were used to calculate the canopy cover area (see Table 1). Annual longitudinal shoot growth was measured in 10 and 5 branches for adults and saplings, respectively. Measurements were made from the 2004 to 2008 cohort based on differences in color and diameter showed by the different cohorts. Needle length was also measured in three needles of each shoot-growth cohort. In 2006 winter, 2004 and 2005 shoot cohorts were measured. In 2007 and 2008 late autumns, 2007 and 2008 shoot cohorts were measured, respectively.

RESISTANCE AND RESILIENCE COMPONENTS

To analyze resistance and resilience to 2005 extreme drought in shoot growth and needle length of considered species, we calculated resistance, recovery, resilience and relative resilience for both variables following the procedure of

Lloret et al. (2011b). Resistance, the inverse of the performance reduction during the extreme drought, is calculated as the ratio between performance during and before drought. Recovery, the ability to recover relative to the performance reduction undergone during drought, is calculated as the ratio between performance after and during the extreme drought. Resilience, the capacity to return to pre-drought performance levels, is calculated as the ratio between the performance before and after drought. Relative resilience is the resilience weighted by the performance reduction during drought, and it is calculated using the following formula:

$$\text{Relative resilience} = (PostDr - Dr) / PreDr$$

where *PreDr*, *Dr* and *PostDr* indicate performance before, during, and after drought, respectively. Performance before, during and after drought were calculated as the average during a two-year period, although this varied due to the species-specific ecological characteristics and sampling limitations. For pines, 2003 and 2004 correspond to pre-drought values; 2005 and 2006 to during-drought values; and 2007 and 2008 to post-drought values. 2005 extreme drought impacted 2005 and 2006 pine shoot cohorts, as the conditions during bud formation affect next year shoot growth (Dobbertin et al. 2010, Isik 1990). For shrubs, 2004 correspond to pre-drought values; 2005 to during-drought values; and 2007 and 2008 to post-drought values. Pre-drought values include only 2004, as the identification of 2003 shoot cohort was not possible in 2006 winter. In contrast to *Pinus*, *Juniperus* presents an indeterminate shoot growth, with only 2005 shoot cohort being affected by the extreme drought. Although only 2007 and 2008 were considered for post-drought values, the inclusion of 2006 values did not change the results.

DATA ANALYSIS

Shoot-growth and needle-length resistance, recovery, resilience, and relative resilience were analyzed to search for differences between species, ontogenetic states (large adults / non-reproductive saplings), and locations (exposure and altitude). Three species comparisons were performed: 1) *P. sylvestris* vs. *J. communis* with a northern exposure at a high elevation; 2) *P. sylvestris* vs. *P. nigra* at a south exposure with a high elevation; and 3) *J. oxycedrus* vs. *P. nigra* at low elevation. For locations, two comparisons were made: 1) between northern and southern exposures for *P. sylvestris*; and 2) between high, medium, and low elevations for *P. nigra*. Differences between species and locations were analyzed using General Linear Mixed Models (GLMM), with species (or location), ontogenetic state and its interaction as fixed factors, and plot as random factor. Shoot growth or needle length resistance, recovery, resilience or relative resilience was the dependent variable in each case. *Post hoc* comparisons between groups were performed using Tukey HSD test. All the analyses were performed using JMP 7.0 (SAS Institute Inc.). All results throughout this paper are given as mean \pm standard error.

RESULTS

SHOOT GROWTH

P. sylvestris vs. *J. communis*

P. sylvestris presented significantly higher resistance but lower relative resilience than did *J. communis* for both adults and saplings (Fig. 1, Table 2). Adult *P. sylvestris* showed slightly negative relative resilience, suggesting an incomplete

Table 2 Summary of GLMM analysis for shoot growth resistance (R_t), recovery (R_c), resilience (R_s), and relative resilience (RR_s) for species and location comparisons. Species comparisons comprise *P. sylvestris* vs. *J. communis*, *P. sylvestris* vs. *P. nigra* and *P. nigra* vs. *J. oxycedrus*. Location comparisons comprise exposure and altitude differences for *P. sylvestris* and *P. nigra*, respectively.

	R_t		R_c		R_s		RR_s	
	F	P	F	P	F	P	F	P
<i>P. sylvestris</i> vs. <i>J. communis</i>								
Species	53.452	<0.0001	52.534	<0.0001	0.820	0.367	51.972	<0.0001
Ontogenetic state (Ont)	0.159	0.690	0.464	0.4968	0.593	0.443	1.486	0.2251
Species x Ont	10.132	0.0018	18.854	<0.0001	0.0001	0.991	7.289	0.0079
<i>P. sylvestris</i> vs. <i>P. nigra</i>								
Species	0.196	0.658	1.145	0.2874	1.637	0.2039	4.077	0.0463
Ontogenetic state (Ont)	0.501	0.481	4.491	0.0367	1.343	0.2494	4.299	0.0408
Species x Ont	4.783	0.031	0.069	0.7928	5.277	0.0238	1.556	0.2153
<i>P. nigra</i> vs. <i>J. oxycedrus</i>								
Species	2.915	0.0902	15.614	0.0001	8.719	0.0038	18.613	<0.0001
Ontogenetic state (Ont)	21.939	<0.0001	20.553	<0.0001	1.148	0.286	17.438	<0.0001
Sp x Ont	18.962	<0.0001	0.112	0.738	5.194	0.0244	0.098	0.754
<i>P. sylvestris</i> : Exposure								
Exposure	0.0001	0.992	0.206	0.651	0.014	0.905	0.013	0.907
Ontogenetic state (Ont)	6.796	0.010	7.623	0.006	0.201	0.655	3.809	0.053
Exposure x Ont	0.013	0.910	1.085	0.300	1.221	0.272	1.099	0.297
<i>P. nigra</i> : Altitude								
Altitude	10.216	<0.0001	0.633	0.532	4.258	0.016	0.244	0.784
Ontogenetic state (Ont)	16.649	<0.0001	27.785	<0.0001	2.359	0.127	25.598	<0.0001
Altitude x Ont	12.081	<0.0001	1.729	0.181	2.947	0.055	0.044	0.956

recovery in shoot growth (Fig. 1, Table 2). On the other hand, *J. communis* adults presented significantly higher recovery than did *P. sylvestris* adults.

P. sylvestris vs. *P. nigra*

Saplings of both species displayed significantly higher recovery and relative resilience than conspecific adults, relative resilience also being significantly higher

in *P. nigra* (Fig. 1, Table 2). Finally, *P. nigra* saplings showed the greatest resilience values (Fig. 1, Table 2).

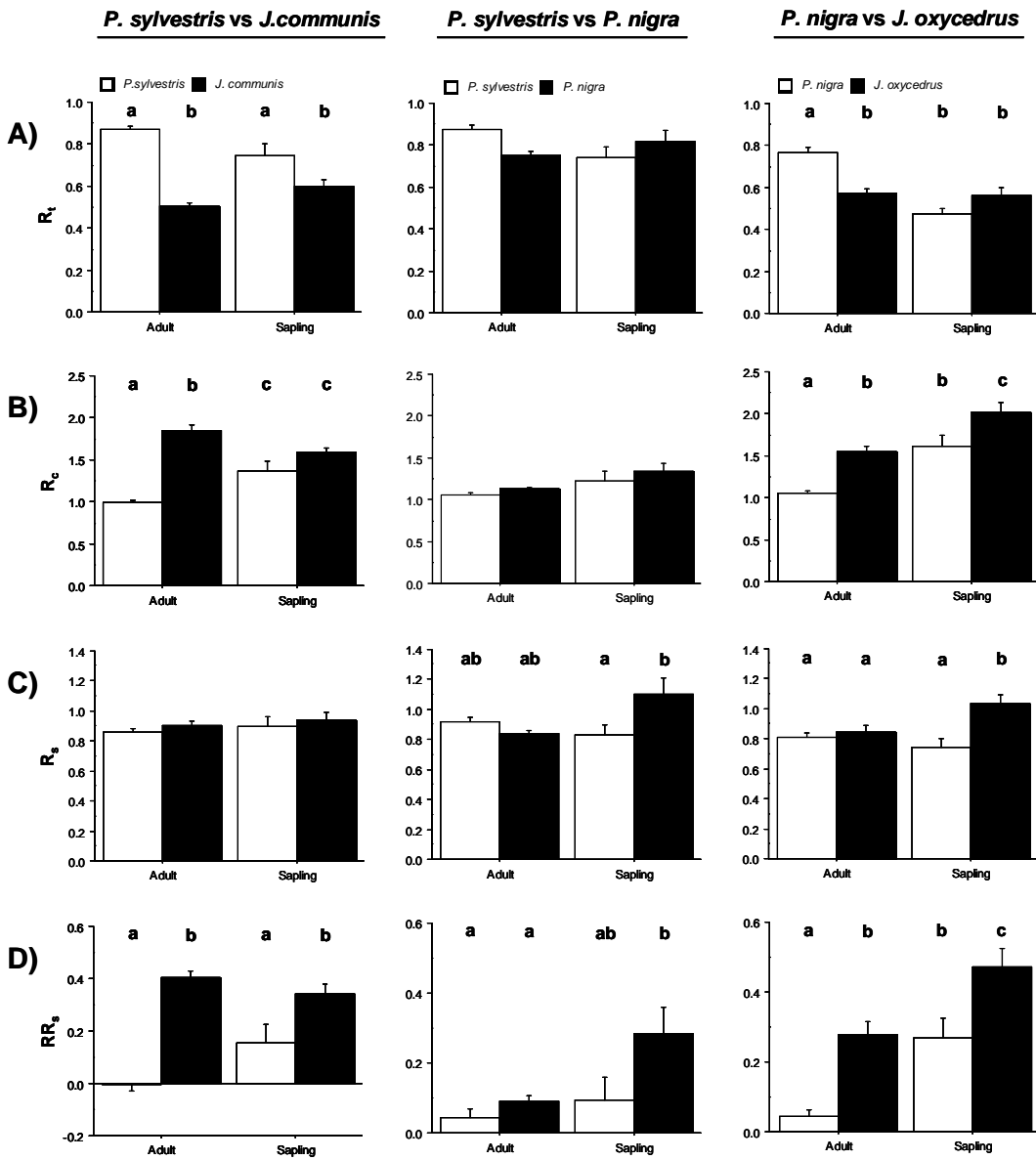


Figure 1 Differences in shoot growth resistance (A), recovery (B), resilience (C) and relative resilience (D) between species and ontogenetic states (adults / saplings). Three comparisons are shown: *P. sylvestris* vs. *J. communis* with a northern exposure at high elevation; *P. sylvestris* vs. *P. nigra* with a southern exposure at high elevation; and *J. oxycedrus* vs. *P. nigra* at low elevation. Different letters above bars indicate significant *post hoc* differences between groups. Bars indicate the standard errors of calculated means.

P. nigra vs. *J. oxycedrus*

J. oxycedrus showed significantly higher recovery and relative resilience than *P. nigra*, values being significantly higher in saplings (Fig. 1, Table 2). *P. nigra* adults showed significantly the highest resistance and *J. oxycedrus* saplings the highest resilience (Fig. 1, Table 2).

P. sylvestris: Exposure

No significant differences were found between exposures in any resistance and resilience components. While adults showed significantly higher resistance than saplings, saplings showed significantly higher recovery (Fig. 2, Table 2).

P. nigra: Altitude

Adults showed significantly lower recovery and relative resilience values than saplings (Fig. 2, Table 2). Differences in altitude appear clearly between saplings, with significantly stronger resistance and resilience at high than at low altitude (Fig. 2, Table 2).

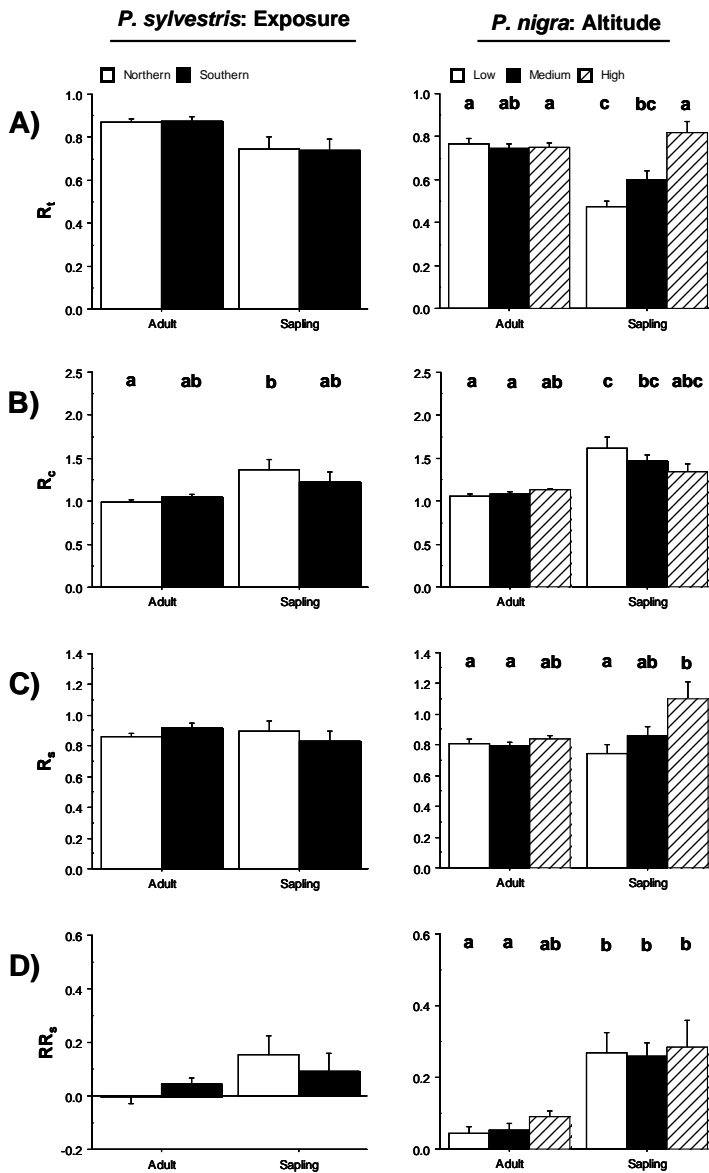


Figure 2 Differences in shoot-growth resistance (A), recovery (B), resilience (C), and relative resilience (D) between locations and ontogenetic states (adults / saplings). Two comparisons are shown: between northern and southern exposure for *P. sylvestris*; and between high, medium, and low elevations for *P. nigra*. Different letters above bars indicate significant *post hoc* differences between groups. Bars indicate the standard errors of calculated means.

NEEDLE LENGTH

P. sylvestris vs. *J. communis*

J. communis showed significantly higher resistance and resilience than *P. sylvestris* for both adults and saplings (Table 3). Furthermore, saplings of *J. communis* displayed significantly the highest recovery (Fig. 3, Table 3).

P. sylvestris vs. *P. nigra*

P. nigra displayed significantly higher recovery and relative resilience than *P. sylvestris*, values being significantly higher in saplings (Fig. 3, Table 3). *P. nigra* also showed significantly higher resilience than did *P. sylvestris* (Table 3).

P. nigra vs. *J. oxycedrus*

J. oxycedrus showed higher resistance but lower recovery and relative resilience than did *P. nigra*, with saplings showing lower resistance but higher recovery and relative resilience (Fig. 3, Table 3). Finally, *J. oxycedrus* showed significantly higher resilience than did *P. nigra* (Table 3).

P. sylvestris: Exposure

P. sylvestris having a northern exposure showed significantly higher recovery and resilience than having a southern exposure, with saplings showing

significant higher recovery (Fig. 3, Table 3). Saplings presented also significantly higher relative resilience than did adults (Table 3).

Table 3 Summary of GLMM analysis for needle-length resistance (R_t), recovery (R_c), resilience (R_s), and relative resilience (RR_s) for species and location comparisons. Species comparisons comprise *P. sylvestris* vs. *J. communis*, *P. sylvestris* vs. *P. nigra* and *P. nigra* vs. *J. oxycedrus*. Location comparisons comprise exposure and altitude differences for *P. sylvestris* and *P. nigra*, respectively.

	R_t		R_c		R_s		RR_s	
	F	P	F	P	F	P	F	P
<i>P. sylvestris</i> vs. <i>J. communis</i>								
Species	21.777	<0.0001	1.947	0.165	32.224	<0.0001	0.020	0.886
Ontogenetic state (Ont)	1.340	0.249	4.297	0.0402	0.159	0.690	1.527	0.219
Species x Ont	0.107	0.743	4.723	0.0316	4.497	0.0359	2.829	0.095
<i>P. sylvestris</i> vs. <i>P. nigra</i>								
Species	0.268	0.606	9.253	0.0031	8.606	0.0043	4.885	0.0298
Ontogenetic state (Ont)	2.888	0.093	10.187	0.0020	2.266	0.136	10.819	0.0015
Species x Ont	0.449	0.505	3.320	0.072	1.055	0.307	0.945	0.333
<i>P. nigra</i> vs. <i>J. oxycedrus</i>								
Species	63.698	<0.0001	74.103	<0.0001	4.1915	0.0427	57.762	<0.0001
Ontogenetic state (Ont)	23.592	<0.0001	49.452	<0.0001	0.075	0.7842	32.525	<0.0001
Sp x Ont	4.296	0.0403	25.462	<0.0001	0.097	0.7553	8.393	0.0045
<i>P. sylvestris</i> : Exposure								
Exposure	1.165	0.283	6.180	0.0147	4.334	0.0401	1.810	0.182
Ontogenetic state (Ont)	1.472	0.228	6.030	0.0159	1.490	0.225	5.816	0.0179
Exposure x Ont	0.007	0.932	0.831	0.364	0.287	0.593	0.231	0.632
<i>P. nigra</i> : Altitude								
Altitude	17.210	<0.0001	9.760	0.0001	1.894	0.154	4.525	0.0125
Ontogenetic state (Ont)	40.032	<0.0001	55.928	<0.0001	0.418	0.519	54.036	<0.0001
Altitude x Ont	2.996	0.0533	2.850	0.0613	0.935	0.395	0.067	0.934

P. nigra: Altitude

P. nigra trees showed significant differences in altitude for resistance, recovery, and relative resilience (Table 3). For resistance, the highest values were for high-elevation individuals and the lowest for low-elevation ones, showing the opposite pattern in the case of recovery and relative resilience. However, differences in elevation were in general more apparent in saplings than in adults.

Saplings showed significantly lower resistance and higher recovery and relative resilience than did adults (Fig. 3, Table 3).

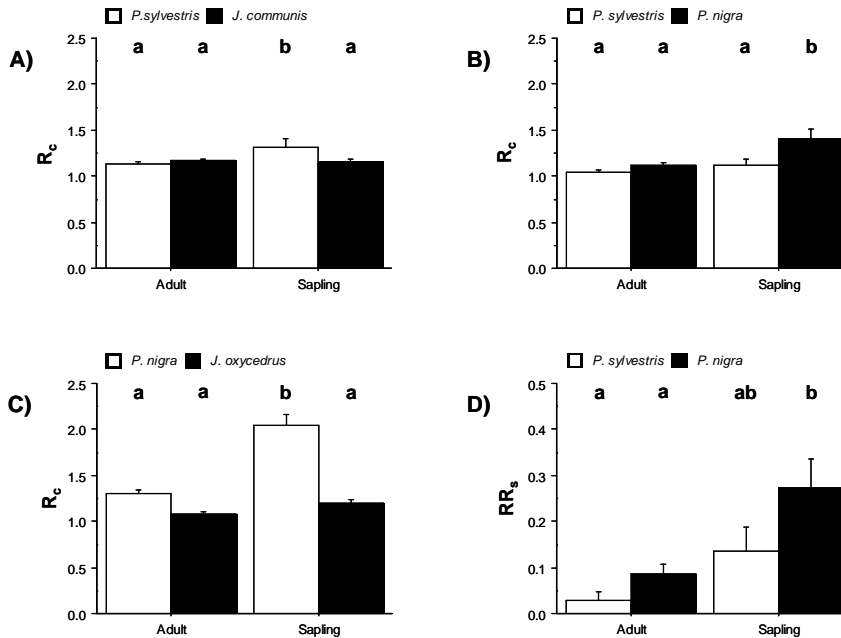


Figure 3 Differences in needle-length recovery for *P. sylvestris* vs. *J. communis* with a northern exposure at high elevation (A), *P. sylvestris* vs. *P. nigra* with a southern exposure at high elevation (B), and *J. oxycedrus* vs. *P. nigra* at low elevation (C) are shown. Differences in needle-length relative resilience for *P. sylvestris* vs. *P. nigra* with a southern exposure at high elevation (D) are also displayed. Different letters above bars indicate significant *post hoc* differences between groups. Bars indicate the standard errors of calculated means.

DISCUSSION

The high vegetative (shoot and leaf growth) resilience values recorded across species, sites and ontogenetic states indicate low impact of the 2005 extreme drought after three years. Thus, it seems that the dominant *Pinus* and *Juniperus* species at their southern distribution edge present great tolerance to extreme drought events. However, resistance and resilience components varied across

species and ontogenetic states. Adult *Pinus* species showed higher shoot growth resistance but lower recovery than adult *Juniperus* species, resulting in similar growth resilience. In general, non-reproductive saplings displayed better growth recovery than conspecific mature adults. *P. nigra* and *J. oxycedrus* saplings at high and low elevation respectively, were the most resilient in terms of growth at all the study locations. With respect to *Pinus*, adults as well as saplings of *P. nigra* displayed higher relative resilience than did those of *P. sylvestris* for shoot growth. On the other hand, resistance and resilience components for needle length responded similarly than did for shoot growth: saplings presented higher recovery than adults and *P. nigra* showed higher relative resilience than *P. sylvestris*.

Contrary to our expectations, adults of both *Juniperus* species showed lower growth resistance (greater reduction of growth) than did *Pinus* adults. We expected the opposite pattern, as *Juniperus* present an anisohydric regulation, allowing higher stomatal conductance and thus higher photosynthetic uptake to be sustained under dry conditions than in isohydric *Pinus* (McDowell et al. 2008, Zweifel et al. 2009). However, the deeper root system of trees presumably provides them access to deeper groundwater, thereby boosting stomatal conductance during the 2005 extreme drought. Nevertheless, adults of *Pinus* and *Juniperus* registered similar growth resilience, due to a tradeoff between recovery and resistance supported by negative correlations between them (data not shown). This tradeoff indicates the importance of the stored reserves in growth resistance and resilience (Galiano et al. 2011, Lloret et al. 2011b). However, *Juniperus* species displayed higher relative resilience than did *Pinus* species, both for saplings and for adults, revealing that *Juniperus* recover for heavier reductions than *Pinus* after 2005 extreme drought.

Significant differences were found among resilience components between large adults and non-reproductive saplings. In general, saplings showed higher recovery than adults for all the exposures and elevations considered. This recovery capacity might be due to higher allocation to shoot growth in saplings than in adults (Day and Greenwood 2011), enhancing growth recovery after the extreme

drought. In addition, *P. nigra* and *J. oxycedrus* saplings at high and low elevations, respectively, were the most resilient in terms of shoot growth. In fact, they were the only cases where shoot growth resilience reached values higher than one, indicating a total recovery after the 2005 extreme drought. In a scenario of recurrent extreme droughts (IPCC 2007, Briffa et al. 2009), higher growth resilience of *P. nigra* and *J. oxycedrus* saplings would have a special relevance through the recruitment advantage.

At low elevation, *J. oxycedrus* would be favored over *P. nigra* due to higher sapling recovery after droughts. This fact, coupled with differential drought sensitivity in adults could induce progressive vegetation shift at generally drier low-elevation forests. In southern *P. nigra* populations, strong declining basal-area increment trends were reported at low elevations, suggesting an intensified drought-induced mortality risk (Linares and Tíscar 2010, 2011). In fact, decline symptoms have been observed in *P. nigra* populations growing at low elevations after the 2005 extreme drought (Linares and Tíscar 2011). However, *Juniperus* adults appear to undergo less mortality risk than do coexisting *Pinus*. In fact, several studies in western USA recorded higher drought-induced mortality of *P. edulis* and *P. ponderosa* in comparison with *J. monosperma* (Allen and Breshears 1998, Floyd et al. 2009, Koepke et al. 2010). Therefore, differences in growth resilience and mortality risk between *P. nigra* and *J. oxycedrus*, could encourage a shift towards a forest with stronger shrub dominance at low elevations, as has been observed in other pine-juniper woodlands (Allen and Breshears 1998, Koepke et al. 2010).

Similarly, higher growth resilience of *P. nigra* saplings than *P. sylvestris* ones can play an important role under a scenario of recurrent extreme droughts. In addition to higher growth resilience of *P. nigra* saplings, *P. nigra* showed higher relative resilience than *P. sylvestris*, both for adults and for saplings. The capacity of *P. nigra* to recover from more severe growth reductions than *P. sylvestris* could be beneficial not only to respond to extreme events, but also to withstand with

progressively warmer and drier conditions. Several studies indicate higher vulnerability to drought for *P. sylvestris* than for *P. nigra* during the ontogeny in locations where the two species coexist (Martinez-Vilalta and Piñol 2002, Castro et al. 2004, Boulant et al. 2008). In fact, in the last few years, drought-induced growth declines and mortality events have been recorded in many southern *P. sylvestris* populations (Martínez-Vilalta and Piñol 2002, Bigler et al. 2006, Thabeet et al. 2009, Galiano et al. 2010). Biotic factors, such as pests or browsing, can exacerbate drought vulnerability, inflicting severe damages (Hódar et al. 2003, Herrero et al. 2011). In the study area, higher ungulate preference for *P. sylvestris* over *P. nigra* reinforced their climatic responses at the treeline, aggravating drought vulnerability of *P. sylvestris* (Herrero et al. 2011). Therefore, higher sapling resilience and overall relative resilience of *P. nigra*, coupled with its lower vulnerability to drought and browsing, could favor a change in dominance toward this Mediterranean species at high elevations.

With regard to differences in growth resilience between ecological conditions, clear patterns were found with elevation between *P. nigra* saplings. High-elevation saplings showed stronger resilience than did the low elevation ones. In addition, *P. nigra* saplings at high elevation displayed higher growth and needle-length resistance than at medium and low elevations. Thus, it seems that the extreme drought impact was stronger at low altitudes, as recorded in other studies (Allen and Breshears 1998, Adams and Kolb 2004, Linares and Tíscar 2010).

Although only saplings of *J. oxycedrus* and *P. nigra*, at low and high elevations respectively, reach growth resilience values greater than one, the rest of values are in general higher than 0.8. Needle-length resilience values were also high, similar to those for shoot-growth resilience (data not shown). This indicates that the impact of the 2005 extreme drought after three years was rather low, confirming our hypothesis that dominant species of Mediterranean pine-juniper woodlands present high tolerance and resilience to extreme droughts at their southern distribution edge. Observed tolerance could be related to plant adaptation

to Mediterranean dry conditions. In fact, high genetic differentiation of southern *P. sylvestris* and *P. nigra* populations (Prus-Glowacki and Stephan 1994, Afzal-Rafii and Dodd 2007), suggest high adaptation to the local environment. For instance, *P. sylvestris* subsp. *nevadensis* showed lower vulnerability to embolism than did other European northern populations (Martinez-Vilalta et al. 2011). The study species might present specific resilience component values above a hypothetical mortality threshold (see Lloret et al. 2011b), as no die-back symptoms were detected. Thus, dominance and maintenance of pine-juniper woodlands in Mediterranean mountains are supported by the remarkable survival ability and longevity of mature individuals (persistence, *sensu* García and Zamora 2003) as well as high tolerance to extreme droughts of adults and saplings.

Our empirical results are also relevant to forecast plant responses and distributional shifts under a climate-change scenario, especially at species distribution limits such as the Mediterranean basin, where extreme events are predicted to be more detrimental and recurrent (Beniston et al. 2007, Lindner et al. 2010). The acclimated response to extreme droughts should be taken in account when species responses are modeled to future climatic conditions, as models predict sharp decreases in plant diversity and performance in Mediterranean mountains (Thuiller et al. 2005, Reich and Oleksyn 2008, Lindner et al. 2010). However, differences in resilience between dominant species as observed in this study for *P. nigra-P. sylvestris* and *P. nigra-J. oxycedrus* within different vegetation belts, can heavily influence vegetation dynamics. Under recurrent extreme droughts, and progressively warmer and drier conditions, such differences can promote changes in both structure and composition of vegetation, even in areas with high tolerance to dry conditions. Thus, a modeling approach to species range shifts clearly requires field studies to analyze plant responses to extreme events over time in dominant species, for an accurate evaluation of the resistance/resilience ability of current vegetation under a climate-change scenario.

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DISCUSIÓN GENERAL

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A lo largo de la presente tesis doctoral hemos analizado la respuesta al estrés ambiental de adultos y juveniles de *P. sylvestris* y *P. nigra* en la montaña mediterránea. En concreto, hemos estudiado la respuesta a eventos de sequía extrema en juveniles y adultos de las dos especies, explorado la relación entre el clima y el crecimiento radial en adultos, y analizado tanto las posibles interacciones entre el clima y la herbivoría, como su importancia relativa en juveniles. Estudios previos realizados en ambientes mediterráneos con plántulas y juveniles de corta edad de diversas especies leñosas han demostrado que la disponibilidad hídrica es el principal factor que limita el establecimiento durante los primeros años (Castro et al. 2002, Gómez-Aparicio et al. 2004, Mendoza et al. 2009, Matías et al. 2012). En cambio la herbivoría por ungulado parece desempeñar un papel secundario en esta fase, probablemente debido al pequeño tamaño de las plántulas y los juveniles de corta edad (Zamora et al. 2001), o a que la supervivencia se concentra en gran parte bajo la cobertura de especies de matorral (Gómez-Aparicio et al. 2004), donde las plántulas/juveniles son menos aparentes para los herbívoros. Una vez superada la fase de plántula y los años posteriores, los juveniles ven incrementado el riesgo de herbivoría hasta alcanzar una altura determinada, en la que el ungulado no alcanza los brotes apicales (en torno a 150 cm, Zamora et al. 2001). El tiempo que necesita el juvenil para alcanzar la altura libre de herbivoría dependerá de su tasa de crecimiento, la cual está a su vez determinada por las principales variables climáticas. La respuesta climática de juveniles y adultos de las especies dominantes que forman el paisaje, es clave a la hora de determinar la dinámica de la comunidad en el actual marco de cambio global. En esta sección discutiremos los resultados obtenidos en los diferentes capítulos en cuanto a la respuesta climática de juveniles y adultos de *P. sylvestris* y *P. nigra*, así como sus posibles interacciones con la herbivoría en la fase pre-reproductora.

RESPUESTA CLIMÁTICA

En la zona de estudio se ha registrado un aumento de las temperaturas y una disminución de las precipitaciones durante los últimos 87 años (*Capítulo 4*), en consonancia con estudios similares (Andreu et al. 2007, Linares et al. 2009, Sánchez-Salguero et al. 2012). Además del incremento de la aridez, que previsiblemente continuará en el futuro, se prevé un aumento de la frecuencia e intensidad de eventos climáticos extremos para la región mediterránea, tales como sequías severas y olas de calor (Beniston et al. 2008, Giorgi & Lionello 2008). Las sequías extremas y las altas temperaturas se asocian a los cada vez más numerosos casos de decaimiento forestal observados en todo el planeta, caracterizados por defoliaciones, reducciones del crecimiento y eventos de mortalidad masiva (Allen et al. 2010, Camarero et al. 2004, Breda et al. 2006), impactos que han sido detectados para *P. sylvestris* y *P. nigra* en el presente trabajo (*Capítulo 1, Capítulo 5*).

Debido al carácter mediterráneo de *P. nigra*, esperábamos una mejor respuesta de esta especie al aumento de la aridez y los eventos de sequía extrema que en la especie boreo-alpina *P. sylvestris*. Son varios los resultados que apuntan hacia una mayor tolerancia a la sequía por parte de *P. nigra* en localidades donde ambas especies coexisten. Los juveniles de *P. nigra* muestran una mayor resiliencia en el crecimiento de rama y la longitud foliar frente a la sequía que los juveniles de *P. sylvestris* (*Capítulo 5*). Además, tanto juveniles como adultos de *P. nigra* muestran una mayor resiliencia relativa en las variables mencionadas que *P. sylvestris*, pudiendo recuperarse de impactos mayores (*Capítulo 5*). En repoblaciones forestales próximas al área de estudio, *P. sylvestris* sufre mayores defoliaciones y reducciones del crecimiento más acusadas que *P. nigra* (Sánchez-Salguero et al. 2012). Finalmente, *P. sylvestris* muestra una mayor mortalidad por sequía que *P. nigra* tanto en juveniles (Boulant et al. 2008, *Capítulo 1*) como en

adultos (Martínez-Vilalta & Piñol 2002). Por lo tanto, *P. sylvestris* se presenta como una especie más vulnerable a la sequía que *P. nigra*.

La mayor vulnerabilidad de *P. sylvestris* a la mortalidad por sequía es de especial interés, ya que los eventos de mortalidad masiva asociados a sequías extremas pueden acarrear importantes cambios en la estructura y composición de los bosques (Allen & Breshears 1998, Koepke et al. 2010). Martínez-Vilalta & Piñol (2002) sugieren que menores tasas de transpiración y gradientes de potencial hídrico más bajos para adultos de *P. nigra*, pueden estar detrás de las diferencias observadas en la mortalidad con *P. sylvestris*. De manera similar, las señales isotópicas del $\delta^{13}\text{C}$ y $\delta^{18}\text{O}$ foliar junto con las tasas de crecimiento en altura, indican un mayor control estomático en juveniles de *P. nigra* frente a las condiciones de sequía extrema que en *P. sylvestris*, registrándose una menor mortalidad para *P. nigra* (Capítulo 1). Así, la mayor sensibilidad climática de *P. nigra*, reduciendo su tasa de transpiración vía control estomático en condiciones de sequía, puede estar desempeñando un papel importante en su menor vulnerabilidad a la mortalidad por sequía.

En línea con los resultados discutidos hasta el momento, en los últimos años se han registrado numerosos casos de decaimiento forestal asociados a sequías en las poblaciones meridionales de *P. sylvestris*, caracterizados por eventos de mortalidad (Martínez-Vilalta & Piñol 2002, Bigler et al. 2006, Galiano et al. 2010) y reducciones del crecimiento (Thabeet et al. 2009, Eilmann et al. 2006, Vilá-Cabrera et al. 2011). A pesar de que también se han observado síntomas de decaimiento en poblaciones de *P. nigra* (Linares & Tíscar 2011, Sánchez-Salguero et al. 2012), el número de casos es menor y están principalmente localizados a baja altitud, donde los efectos de la sequía son generalmente más acusados (Allen & Breshears 1998, Adams & Kolb 2004, Dobbertin et al. 2005). Así, podemos esperar una disminución paulatina de *P. sylvestris* en el límite sur de distribución más afectado por las sequías, y un aumento de la dominancia de *P. nigra* en las zonas donde ambas especies coexisten.

El estudio de la relación clima-crecimiento en el ámbito de la dendrocronología ha sido ampliamente usado para analizar la respuesta climática de poblaciones arbóreas. Debido a que las poblaciones situadas en el límite inferior de distribución están fuertemente limitadas por la sequía, podríamos esperar una gran influencia de la precipitación y la temperatura estival en el crecimiento, especialmente en *P. sylvestris*. Sorprendentemente, los adultos de *P. sylvestris* y *P. nigra* apenas presentan señales de precipitación en la relación clima-crecimiento en la zona de estudio (*Capítulo 4*), siendo este patrón mas propio de zonas mas septentrionales (Andreu et al. 2008). Estos resultados contrastan con la gran influencia de la precipitación en el crecimiento secundario detectada en otras poblaciones de ambas especies limitadas por la sequía (Martín-Benito et al. 2010, Weber et al. 2007, Eilman et al. 2009, Affolter et al. 2010). De manera similar, en repoblaciones forestales próximas al área de estudio, tanto *P. sylvestris* como *P. nigra* presentan fuertes señales de precipitación en su crecimiento (Sánchez-Salguero et al. 2012). Los factores detrás de la escasa influencia de la precipitación en el área de estudio en comparación con otras localidades pueden ser de diversa índole: microclimáticos, genéticos, y los relacionados con la estructura del bosque. El estudio de la influencia de estos factores en la respuesta poblacional a los rápidos cambios climáticos, puede ser de gran valor a la hora de predecir los posibles cambios en la distribución y composición de las comunidades vegetales.

Es muy posible que las condiciones microclimáticas mitiguen el impacto de las sequías en la zona de estudio. La compleja orografía propia de las montañas mediterráneas posibilita la existencia de condiciones ambientales muy diferentes en distancias relativamente pequeñas. De hecho, se observa un descenso de las temperaturas medias y máximas, así como un aumento de la humedad relativa, con la altitud (*Capítulo 4*). Además, la alta frecuencia y persistencia de nieblas en las partes más altas de la zona de estudio (*observación personal del autor*), puede suponer un aporte hídrico adicional así como una disminución de las tasas de transpiración. Por lo tanto, las poblaciones situadas a mayor altitud sufrirán un

menor impacto de las sequías que las situadas a altitudes más bajas. De hecho, la población de *P. nigra* situada en el límite inferior altitudinal es la única que muestra una señal fuerte de la precipitación en el crecimiento (*Capítulo 4*). Además, las poblaciones situadas a altitudes bajas y medias muestran un incremento de la variabilidad en el crecimiento radial junto al aumento de la aridez, tendencia que no se observa para las poblaciones más altas (*Capítulo 4*). De manera similar, los juveniles de *P. nigra* situados en límite superior muestran una mayor resistencia y resiliencia en el crecimiento de rama frente a condiciones de sequía extrema que los situados en el límite inferior (*Capítulo 5*). Estos resultados ponen de relieve la importancia de las condiciones climáticas a pequeña escala en la respuesta ambiental de las especies.

Por otro lado, una alta adaptación a las condiciones locales puede estar influenciando la relación clima-crecimiento. Las poblaciones naturales de *P. sylvestris* y *P. nigra* más meridionales de la Península Ibérica han estado aisladas durante largo tiempo de las poblaciones situadas más al norte, dando lugar a una alta diferenciación genética (Prus-Glowacki and Stephan 1994, Afzal-Rafii and Dodd 2007). La diferenciación genética es una característica común en las poblaciones relictas (Hampe & Petit 2005), dónde la selección favorable a la adaptación local es mas probable que en otras zonas (Dynesius & Jansson 2000). Así, la subespecie *nevadensis* de *P. sylvestris* propia de la zona de estudio presenta una vulnerabilidad al embolismo menor que otras poblaciones septentrionales (Martinez-Vilalta et al. 2009). En un estudio experimental realizado con semillas de *P. sylvestris* de origen centro-europeo y mediterráneo, éstas últimas mostraron una mayor emergencia y supervivencia bajo diferentes patrones de precipitación (Richter et al. 2011). Sin embargo, una alta diferenciación genética suele estar asociada a una baja variabilidad genotípica en las poblaciones relictas (Hampe & Petit 2005), la cual puede ser perjudicial para su persistencia ante condiciones cambiantes. Por consiguiente, parece necesario tener en cuenta los factores

genéticos y de adaptación local a la hora de predecir la respuesta ambiental de las poblaciones situadas en el límite inferior de distribución.

Por último, factores relacionados con la estructura del bosque pueden afectar de manera determinante la respuesta ambiental de las especies arbóreas. La notable influencia de la precipitación en el crecimiento (Sánchez-Salguero et al. 2012), contrasta con los resultados obtenidos en las poblaciones naturales, donde no se han detectado señales fuertes de la precipitación en el crecimiento (*Capítulo 4*). Las repoblaciones forestales mencionadas se caracterizan por altas densidades de individuos adultos, más del doble que en las poblaciones naturales (Sánchez-Salguero et al. 2012a, *Capítulo 4*). Esta alta densidad, debida a la falta de actuaciones de aclareo de gran coste económico, aumenta la competencia por los recursos hídricos provocando un incremento de la vulnerabilidad al estrés hídrico (Linares et al. 2009, Vilá-Cabrera et al. 2011, Moreno-Gutiérrez et al. 2011, Martínez-Vilalta et al. 2012). Los tres factores comentados hasta el momento (microclima, genética y densidad) pueden haber influenciado en parte la buena recuperación observada en el crecimiento de rama y la longitud de acícula en los pinares de montaña mediterránea después de la sequía extrema de 2005 (*Capítulo 5*). En esta línea, el estudio de los factores y mecanismos que aumentan la resiliencia de las poblaciones en el límite inferior latitudinal, puede ayudar a obtener información que permita un mejor manejo de los ecosistemas forestales, con el fin de aumentar su resiliencia ante los impactos de los rápidos cambios climáticos.

Aparte de la respuesta a la precipitación, la respuesta a la temperatura puede jugar un papel fundamental, ya que las altas temperaturas pueden exacerbar el efecto negativo de la sequía (Adams et al. 2009). La sequía extrema de 2005 asociada a la mortalidad masiva de juveniles de *P. sylvestris* y *P. nigra* en Sierra Nevada, estuvo caracterizada por un verano excepcionalmente caluroso (*Capítulo 1*). Las altas temperaturas aumentan los gastos de carbono debido a tasas más altas de respiración y reparación celular (Hartley et al. 2006, Atkin & Macharel 2009),

incrementando el riesgo de mortalidad por sequía (McDowell et al. 2012). En este sentido, es muy posible que el aumento de las temperaturas junto al incremento de la frecuencia de las sequías extremas conlleve un aumento de los eventos de mortalidad masiva (Adams et al. 2009).

Por otro lado, tanto *P. sylvestris* como *P. nigra* presentan efectos negativos de la temperatura en el crecimiento radial, particularmente de la temperatura de Septiembre del año previo (*Capítulo 5*). Este efecto negativo ha sido detectado anteriormente en otras poblaciones de *P. sylvestris* y *P. nigra* (Rigling et al. 2002, Andreu et al. 2007, Martín-Benito et al. 2010), y se debe probablemente a la prolongación de la sequía estival propia del clima mediterráneo. En este sentido, es posible que el aumento de las temperaturas determine el estrés hídrico durante el otoño tanto o más que la disminución en las precipitaciones.

Si bien el aumento de las temperaturas en otoño tiene un efecto negativo en el crecimiento radial de los adultos de *P. sylvestris* y *P. nigra*, el aumento de las temperaturas de invierno y primavera tiene un efecto positivo (*Capítulo 4*). En el caso de *P. nigra*, una especie con un carácter mas térmico que *P. sylvestris*, temperaturas invernales mas altas pueden prevenir posibles daños por heladas. En contra, las temperaturas altas en primavera posiblemente permitan adelantar el inicio de la temporada de crecimiento (Richter & Eckstein 1990). Por lo tanto, el balance entre los efectos positivos y negativos de la temperatura puede determinar en gran parte la futura respuesta de las poblaciones de *P. sylvestris* y *P. nigra* a los rápidos cambios climáticos.

EFFECTOS DE LA HERBIVORÍA

La herbivoría es, junto al clima, uno de los principales factores que determinan la distribución, abundancia y crecimiento de las especies vegetales (Davidson 1993, Gill et al. 2006, Speed et al. 2010). Los juveniles de *P. sylvestris*

y *P. nigra* parecen recibir mas daños de herbivoría por parte de ungulados que de insectos en el límite forestal (*Capítulo 2*). La baja densidad de juveniles de pino en el límite forestal, que puede dificultar el movimiento de los insectos entre árboles (Dalin et al. 2009), y las bajas temperaturas propias de estas zonas, que condicionan fuertemente la fisiología de los insectos herbívoros (Bale et al. 2002), han podido limitar el impacto de los insectos en los juveniles del límite forestal. Sin embargo, en las zonas de bosque tampoco se han detectado grandes daños por insectos (*observación personal del autor*). Aún así, la procesionaria del pino (*Thaumetopoea pityocampa* Den. et Schiff.) puede reducir significativamente el crecimiento en altura en los juveniles de pino en zonas de bosque (Hódar et al. 2003), pero durante los años de estudio la incidencia de esta plaga ha sido relativamente baja (Hodár & Zamora 2009). En cambio, los ungulados producen daños de manera más constante, impactando negativamente el crecimiento en altura de los juveniles a largo plazo (*Capítulo 3*). Además, las poblaciones de ungulados silvestres han aumentado sensiblemente sus poblaciones en los últimos años, así como los daños producidos (Cote 2004, *Capítulo 3*). Por lo tanto, el estudio del impacto de la herbivoría por ungulado en el crecimiento y demografía de las especies arbóreas es de gran importancia en el actual contexto de cambio global.

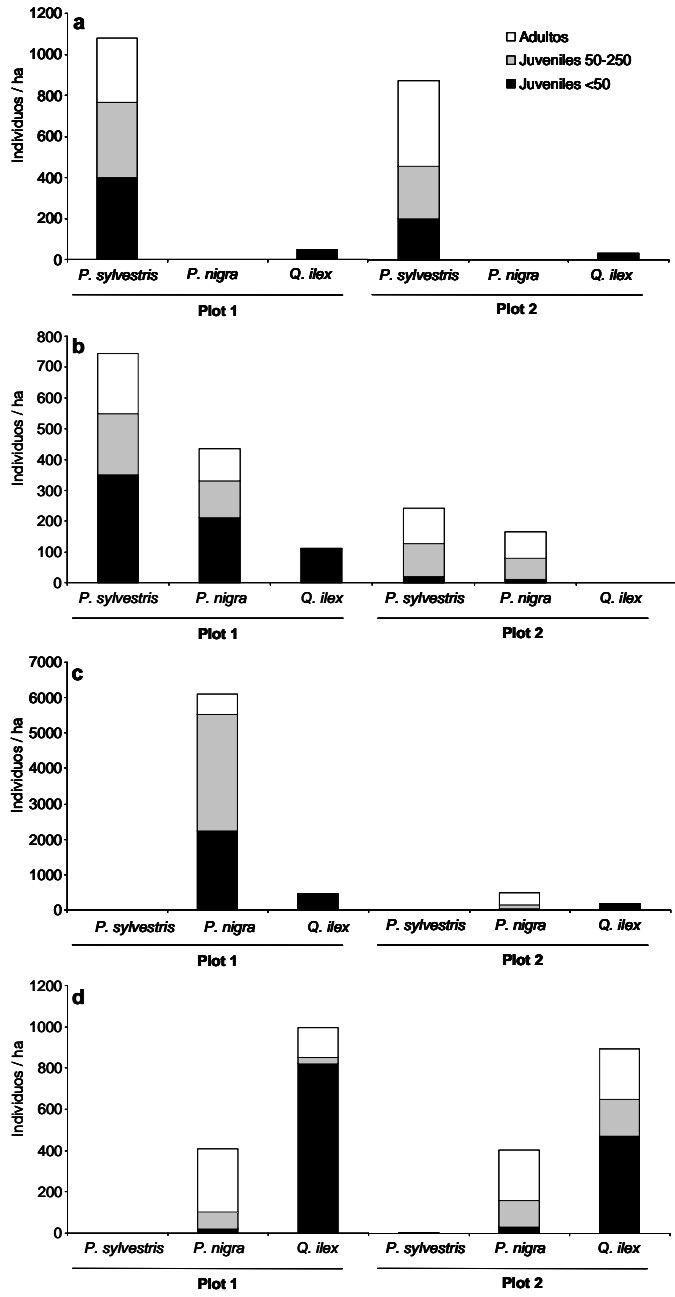
Los ungulados muestran una preferencia clara por *P. sylvestris* en comparación con *P. nigra* (*Capítulo 2, 3*), probablemente debido a su mayor contenido proteico y menor contenido en fibra (Baraza et al. 2009). Castro et al. (2004) registraron mayores daños para juveniles de corta edad de *P. nigra* que de *P. sylvestris*, aunque el resultado de este estudio puede estar influenciado por el mayor tamaño de los individuos de *P. nigra*, resultando mas aparentes para los herbívoros (Baraza 2004). La herbivoría produce un impacto negativo en el crecimiento en altura de los juveniles, tanto en el límite forestal como en las zonas de bosque (*Capítulo 2, 3*). Esta reducción del crecimiento aumenta a su vez el riesgo de herbivoría, y retrasa el inicio de la edad reproductora (Zamora et al. 2001). *P. nigra* alcanza la edad reproductora más tarde que *P. sylvestris*, pero una

vez la alcanza, el número de vástagos producidos respecto a su edad es mayor que en *P. sylvestris* (Debain et al. 2007, Boulant et al. 2008). Por lo tanto, la preferencia de los ungulados por *P. sylvestris* produce un retraso en la edad reproductora en un número más elevado de juveniles que en *P. nigra*, pudiendo provocar que *P. sylvestris* pierda una de las ventajas que tiene sobre *P. nigra*. De esta manera, la herbivoría por ungulado puede influir significativamente en la demografía de las dos especies.

RESPUESTA DEMOGRÁFICA

La mayor vulnerabilidad a la sequía y a los daños por ungulados de *P. sylvestris* en comparación con *P. nigra* puede tener consecuencias demográficas en las localidades donde ambas especies coexisten. Además, debido a la proximidad de muchas de sus poblaciones, podemos esperar también una expansión de *P. nigra* hacia zonas ocupadas por *P. sylvestris*, generalmente más elevadas y/o húmedas (Blanco et al. 1997). Para comprobar estos posibles cambios, a continuación discutiremos datos demográficos de las principales especies arbóreas en el área de estudio, correspondientes a las parcelas muestreadas en el *Capítulo 5*. Estos datos se exponen en las Figuras 1 y 2.

P. sylvestris y *P. nigra* coexisten en las exposiciones sur situadas a 2000 m de altitud. Estos bosques mixtos están dominados por *P. sylvestris*, que muestra una densidad de adultos mayor que la de *P. nigra* en las dos parcelas (Fig. 1). Sin embargo, el índice de regeneración (IR; la capacidad de reemplazamiento de los adultos por los juveniles, Hogg & Schwarz 1997) es similar para las dos especies. Por lo tanto, en el futuro podemos esperar una reducción de la dominancia de *P. sylvestris* en favor de *P. nigra* en el estrato arbóreo. Estos resultados contrastan con las zonas orientadas al norte, donde la dominancia de *P. sylvestris* es total. Por lo



tanto, es posible que *P. nigra* aumente su presencia respecto a *P. sylvestris* en las zonas más soleadas, probablemente más favorables para la especie mediterránea.

P. nigra es la especie dominante en el estrato arbóreo a altitudes medias (1700 m, Fig. 1), presentando el mayor IR también a esta altitud (Fig. 2). En cambio, a altitudes bajas (1500 m) comparte la dominancia con *Quercus ilex*, mostrando *P. nigra* una densidad de adultos mayor o similar. Sin embargo, *Q. ilex* muestra un mayor IR que *P. nigra*, indicando un futuro aumento de *Q. ilex* en el estrato arbóreo. En conjunto, estos datos indican una paulatina migración altitudinal de *P. nigra*, con una tendencia a aumentar su presencia en las cotas más altas y a disminuirla en las cotas más bajas.

Cabe destacar la disparidad del IR entre algunas de las parcelas situadas en la misma exposición o cota altitudinal, como el caso de *P. sylvestris* en exposiciones norte a cotas altas y el de *P. nigra* a altitudes medias. Los IR bajos obtenidos en una de las dos parcelas muestreadas parecen asociados a un menor número de claros desprovistos de cobertura arbórea, donde se localizan gran parte de los juveniles de ambas especies, debido a su baja tolerancia a las condiciones de sombra. Además, el número de juveniles registrado no es especialmente bajo (Fig. 1), y el reemplazamiento de los adultos no parece peligrar.

Hay que tener en cuenta que las predicciones basadas en el IR asumen una ausencia de mortalidad en juveniles (Hogg & Schwarz 1997). Sin embargo, los juveniles de *P. sylvestris* y *P. nigra* pueden sufrir procesos de mortalidad masiva asociados a sequías extremas (Capítulo 1). La mayor mortalidad por sequía de *P. sylvestris* podría, en un escenario con alta frecuencia de sequías extremas, acelerar el cambio de dominancia a favor de *P. nigra* en las exposiciones sur a cotas altas. Además de la menor vulnerabilidad a la sequía y a los ungulados de *P. nigra*, esta especie muestra una mayor tasa de reproducción neta y una mayor dispersión que *P. sylvestris* (Debain et al. 2007, Boulant et al. 2008), lo que puede facilitar su expansión altitudinal en detrimento de *P. sylvestris*.

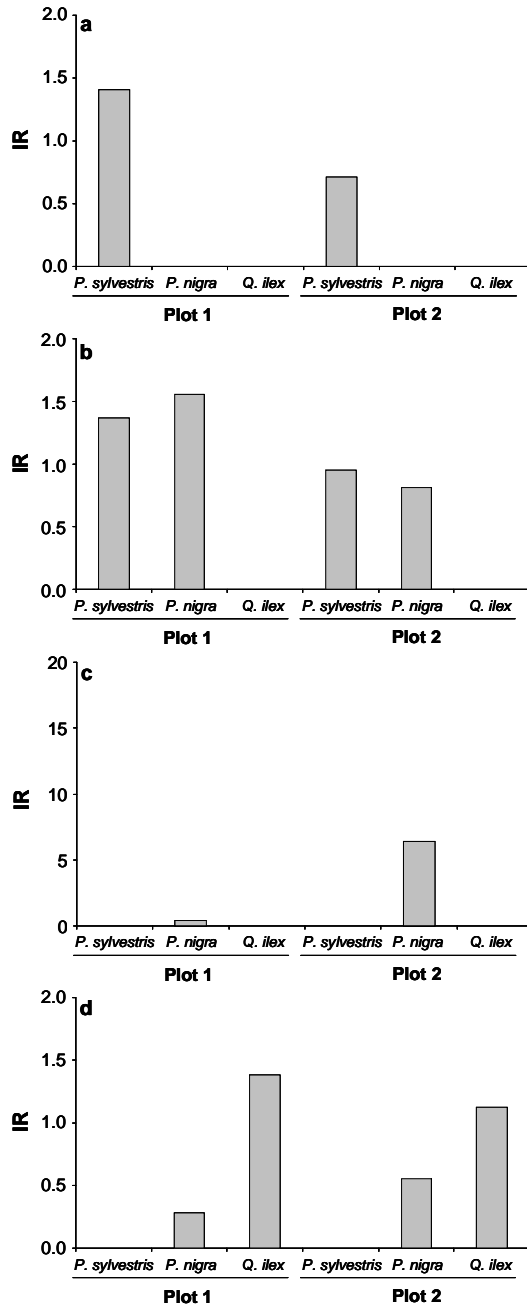


Figura 2 Índice de regeneración (IR) calculado para las principales especies arbóreas en la Sierra de Baza a lo largo de un gradiente ambiental de altitud y exposición: 2000 m norte (a), 2000 m sur (b), 1700 m norte (c) y 1500 m norte (d). El IR se ha calculado dividiendo el número total de juveniles entre el número de adultos (Hogg y Schwarz 1997). IR = 1 indica un futuro reemplazamiento de los adultos; IR <1 indica una menor capacidad de regeneración; IR >1 indica una mayor capacidad de regeneración. Este índice se calculó sólo si el número de individuos adultos de la especie en cuestión representaba más del 2% del total de adultos, descartando por tanto las especies arbóreas con escasa representación de adultos en cada una de las parcelas.

INTERACCIONES CLIMA-HERBIVORÍA Y SU IMPORTANCIA RELATIVA

La herbivoría puede influenciar enormemente la respuesta de las comunidades vegetales al calentamiento global (Post & Pedersen 2008). Así, los desplazamientos altitudinales y latitudinales en el frente de expansión pueden verse bloqueados debido a los daños producidos por la herbivoría (Oloffson et al. 2009, Speed et al. 2010). La herbivoría puede también exacerbar los efectos de los cambios climáticos, produciendo daños importantes en especies con una alta vulnerabilidad a las nuevas condiciones climáticas. Este es el caso de *P. sylvestris*, mas vulnerable a la sequía que *P. nigra*, que sufre mayores daños por herbivoría en el límite forestal (*Capítulo 2*). Además, *P. sylvestris* es incapaz de compensar los daños sufridos con una mayor tasa de crecimiento en altura (*Capítulo 2*). Debido a la importancia de las interacciones entre la herbivoría y el clima, discernir el efecto relativo de ambos factores puede resultar crítico a la hora de predecir cambios en la abundancia y distribución de las especies en el contexto del cambio global.

La precipitación y la herbivoría por ungulado se postulan como los principales factores que determinan el crecimiento en altura a largo plazo en juveniles de *P. sylvestris* y *P. nigra* (*Capítulo 3*). Tanto el efecto positivo de la precipitación como el negativo de la herbivoría se sobreestiman al agregar los datos individuales a la escala de paisaje. Así, nuestros resultados demuestran que la respuesta a la herbivoría y al clima ocurre mayormente a escala individual, y que la agregación de los datos individuales acarrea una degradación de la información al relegar a un segundo plano la escala de interés (*Capítulo 3*). Por lo tanto, una aproximación a nivel individual del efecto de la precipitación y la herbivoría en el crecimiento permite una mejor cuantificación de su importancia relativa.

El impacto de la precipitación y la herbivoría en el crecimiento de *P. sylvestris* y *P. nigra* a escala individual es similar en zonas de bosque, no así en el límite forestal, donde el impacto de la precipitación en el crecimiento de *P. sylvestris* es mayor (*Capítulo 3*). Esto puede deberse a que el límite forestal es una

zona con una menor disponibilidad hídrica debido a la escorrentía causada por una mayor pendiente que en las zonas de bosque, y a un mayor efecto desecante del viento a mayores altitudes. Por lo tanto, el efecto relativo de la herbivoría y la precipitación puede variar espacialmente, y el conocimiento de esta variabilidad puede ser de gran ayuda a la hora de predecir la respuesta de las especies al incremento de la aridez y de las poblaciones de ungulado.

La aproximación individual realizada en el *Capítulo 3* nos permite también explorar los patrones del efecto de la precipitación y la herbivoría en el crecimiento en altura. La herbivoría presenta un efecto más variable que la precipitación, con algunos individuos muy dañados que concentran el consumo de los ungulados, mientras otros apenas sufren daños. En cambio la precipitación tiene un efecto más homogéneo y extensivo en la población, sincronizando la respuesta en el crecimiento en el límite forestal, dónde su influencia es mayor. Así pues, a pesar de que la respuesta a la precipitación y la herbivoría ocurre a nivel individual, la precipitación opera a una escala más amplia, mientras que la herbivoría se concentra en los individuos mas dañados.

SÍNTESIS

En la Figura 3 se muestra un resumen de los resultados obtenidos en el presente trabajo. Hemos estudiado las diferencias en la vulnerabilidad a la sequía y la herbivoría desde una perspectiva multidisciplinar, analizando variables ecológicas, ecofisiológicas, y demográficas. Ambas especies muestran crecimientos en altura e índices de regeneración similares en localidades donde coexisten. Sin embargo, los juveniles de *P. nigra* muestran una mayor resiliencia en el crecimiento de rama y longitud foliar, así como una menor mortalidad por sequía, probablemente debido a un mejor ajuste de la eficiencia en el uso del agua. Además, los herbívoros muestran una menor preferencia por *P. nigra*, que sufre

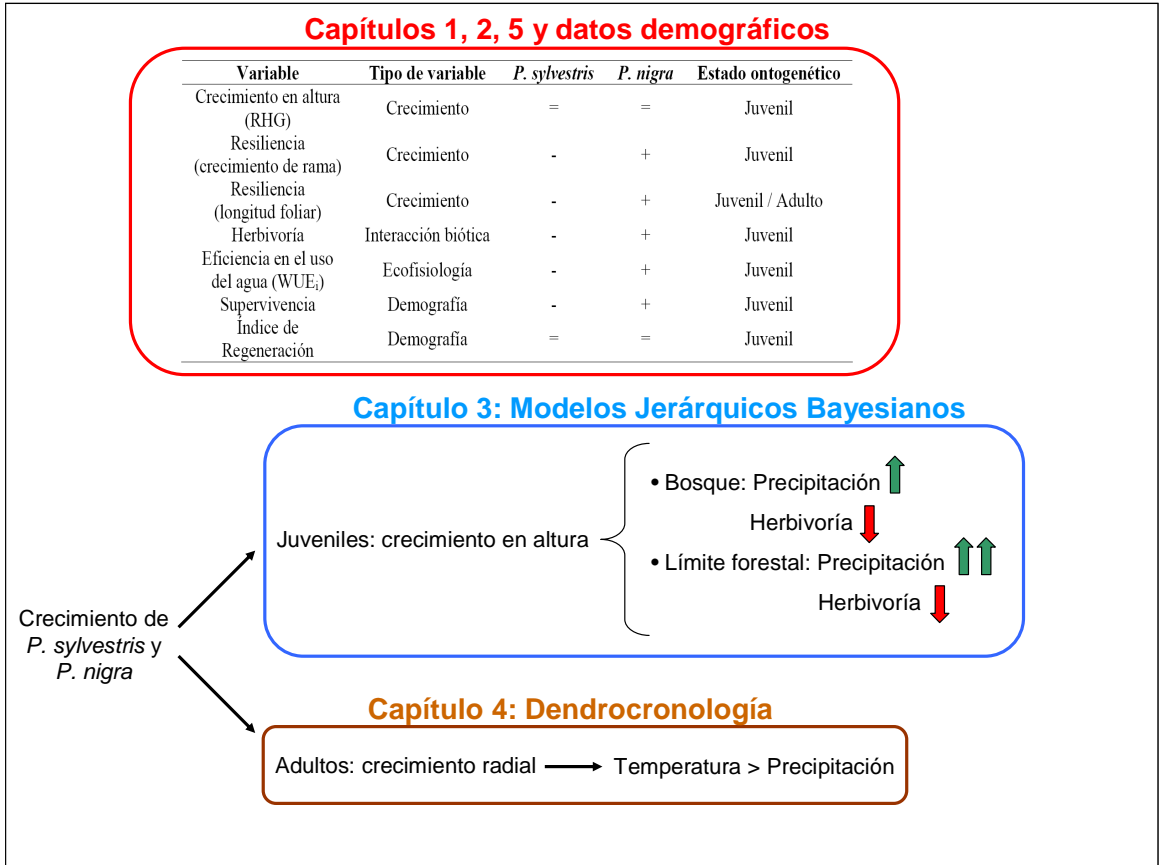


Figura 3 Resumen de los resultados obtenidos en la presente tesis doctoral. En la tabla de la parte superior de detalla la comparación entre las dos especies para diferentes variables de crecimiento, de interacción biótica, ecofisiológicas, y demográficas. El signo (+) indica una ventaja para alguna de las dos especies bajo un escenario de incremento de la aridez, el (-) una desventaja, y el (=) una ausencia de diferencias remarcables. En la parte inferior se presentan las principales influencias para el crecimiento en juveniles y adultos de ambas especies. Una flecha verde indica un efecto positivo en el crecimiento, dos flechas verdes un efecto positivo mayor, y una flecha roja un efecto negativo.

menores daños que *P. sylvestris*. Por lo tanto, el balance es claramente desfavorable para la especie boreal en un escenario de cambio global, con una más que posible recurrencia de sequías extremas, y una gran población de ungulados silvestres.

El crecimiento es la tasa demográfica más estudiada en el presente trabajo, tanto en juveniles como en adultos (Fig. 3). El crecimiento en altura en individuos juveniles está determinado fundamentalmente por la precipitación y la herbivoría por ungulado, siendo la herbivoría tan importante como la precipitación en las

zonas de bosque. Por lo tanto, nuestros resultados revelan que la herbivoría juega un papel fundamental en el crecimiento a largo plazo de los juveniles pre-reproductores, los cuales representan el futuro inmediato del bosque. En cambio, el crecimiento radial de los adultos está influenciado en mayor medida por la temperatura que por la precipitación. La gran influencia de la temperatura es un resultado de gran relevancia debido a los importantes cambios que se esperan en las temperaturas (Trenberth et al. 2007). El balance de los efectos positivos y negativos de la temperatura será decisivo en el futuro de las poblaciones de *P. sylvestris* y *P. nigra* situadas en el límite sur de distribución.

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CONCLUSIONES / CONCLUSIONS

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1. *P. nigra* presenta una menor mortalidad por sequía que *P. sylvestris* en su fase pre-reproductora. El mayor control estomático de *P. nigra* frente a las condiciones de sequía puede ser una de las causas determinantes de su menor vulnerabilidad a la mortalidad por sequía.
2. La herbivoría por ungulado produce en los juveniles de *P. sylvestris* y *P. nigra* daños severos de manera más constante que la ocasionada por los insectos. Los ungulados muestran una mayor preferencia por *P. sylvestris*, reduciendo el crecimiento en altura y retrasando el inicio de la edad reproductora en los juveniles dañados.
3. La herbivoría por ungulado refuerza la respuesta climática de juveniles de *P. sylvestris* y *P. nigra* en el límite forestal, provocando más daños en *P. sylvestris*, especie más vulnerable a la sequía que no es capaz de compensar los daños por herbivoría con una mayor tasa de crecimiento en altura.
4. La herbivoría por ungulado y la precipitación son los principales factores que determinan el crecimiento en altura a largo plazo en juveniles de *P. sylvestris* y *P. nigra*. La respuesta a la herbivoría y a la precipitación ocurre mayormente a escala individual, y la agregación de los datos individuales a escala de paisaje provoca una sobreestimación del impacto de la herbivoría y la precipitación.
5. El impacto negativo de la herbivoría y el positivo de la precipitación en el crecimiento de *P. sylvestris* y *P. nigra* a escala individual es similar en zonas de bosque, no así en el límite forestal, donde el impacto de la precipitación en el crecimiento de *P. sylvestris* es mayor. Mientras que la herbivoría presenta un efecto más variable que la precipitación, con algunos individuos muy dañados que concentran el consumo de los ungulados, la precipitación tiene un efecto más homogéneo y extensivo en la población.

6. El crecimiento radial en adultos de *P. sylvestris* y *P. nigra* en límite sur de distribución está influenciado en mayor medida por la temperatura que por la precipitación. El balance entre el efecto negativo de las temperaturas de otoño y el positivo de las temperaturas de invierno y primavera determinará en gran parte la persistencia de estas poblaciones en el futuro.
7. Los juveniles de *P. nigra* muestran una mayor resiliencia en el crecimiento de rama y hoja que los juveniles de *P. sylvestris* frente a eventos de sequía extrema.
8. La ausencia de señales fuertes de precipitación en el crecimiento radial de *P. sylvestris* y *P. nigra*, y la resiliencia relativamente alta observada en el crecimiento de rama de adultos y juveniles de las dos especies frente a un evento de sequía extrema puede estar relacionada con procesos de compensación demográfica. Las condiciones climáticas a escala local, la adaptación local asociada a una alta diferenciación genética, y la baja densidad arbórea pueden mitigar el impacto de las sequías en las poblaciones de *P. sylvestris* y *P. nigra* situadas en límite sur de distribución.
9. Debido a la mayor vulnerabilidad a la sequía y a la herbivoría por parte de *P. sylvestris* en el actual escenario de cambio global, podemos esperar un cambio de dominancia a favor de *P. nigra* en zonas dominadas hoy en día por *P. sylvestris*.

CONCLUSIONS

1. *P. nigra* shows lower drought mortality than *P. sylvestris*. Tighter control of stomatal conductance during drought conditions by *P. nigra* may play an important role in its lower vulnerability to drought mortality.
2. Ungulate herbivory produce severe damages in *P. sylvestris* and *P. nigra* saplings more continuous than do insects. Ungulates showed higher preference by *P. sylvestris*, reducing height growth and delaying reproductive age in browsed saplings.
3. Ungulate herbivory reinforces climatic response of *P. sylvestris* and *P. nigra* saplings in the treeline, producing more damages in *P. sylvestris*, which is a species more vulnerable to drought unable to compensate the herbivory damages with higher height growth rate.
4. Ungulate herbivory and precipitation are the main factors determining long-term height growth in *P. sylvestris* and *P. nigra* saplings. The response to herbivory and precipitation occur mainly at individual level, and aggregation of individual data in landscape scale produce an overestimation of herbivory and precipitation impacts.
5. The positive impact of herbivory and negative of precipitation in the height growth of *P. sylvestris* and *P. nigra* saplings at individual level is similar in forest areas, but not in treeline, where the impact of precipitation in *P. sylvestris* growth is higher. While herbivory shows a more variable effect than precipitation, with some highly damaged individuals concentrating ungulate consume, precipitation effect is more homogeneous and pervasive in the population.
6. Radial growth in *P. sylvestris* and *P. nigra* adults in the southern distribution limit is more determined by temperature than by precipitation. The balance between negative effect of high autumn temperatures and

positive of high winter and spring temperatures could determine in part the future persistence of these populations.

7. *P. nigra* saplings show higher shoot and leaf growth resilience than *P. sylvestris* saplings to extreme drought events.
8. The absence of strong precipitation signals in *P. sylvestris* and *P. nigra* radial growth, and the relative high shoot growth resilience observed in adults and saplings of the two species to an extreme drought event could be related to demographic stabilizing processes. Local climatic conditions, local adaptation linked to high genetic differentiation, and low tree density could diminish the impact of drought conditions in *P. sylvestris* and *P. nigra* populations located in the southern distribution limit.
9. Due to higher vulnerability to drought- and ungulate-induced damages of *P. sylvestris*, we can expect a change in dominance toward *P. nigra* in areas dominated by *P. sylvestris* until nowadays.