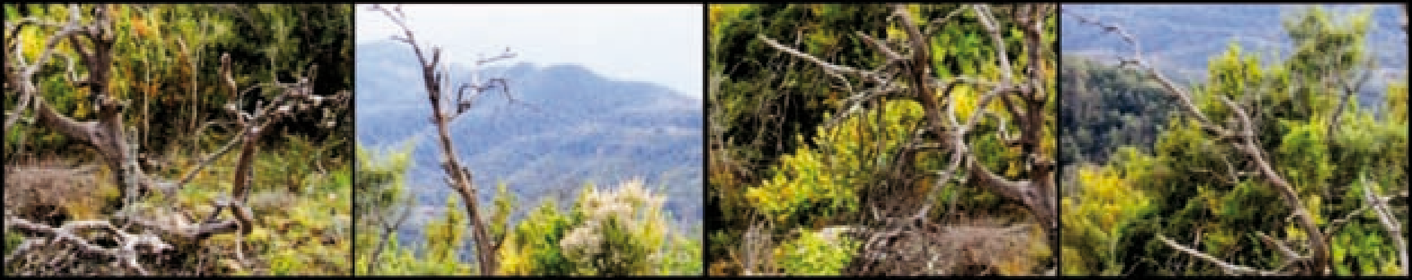
Drought-induced die-off in *Pinus sylvestris* and *Quercus ilex* forests of Catalonia



Lucía Galiano Pérez • Tesis doctoral • 2012

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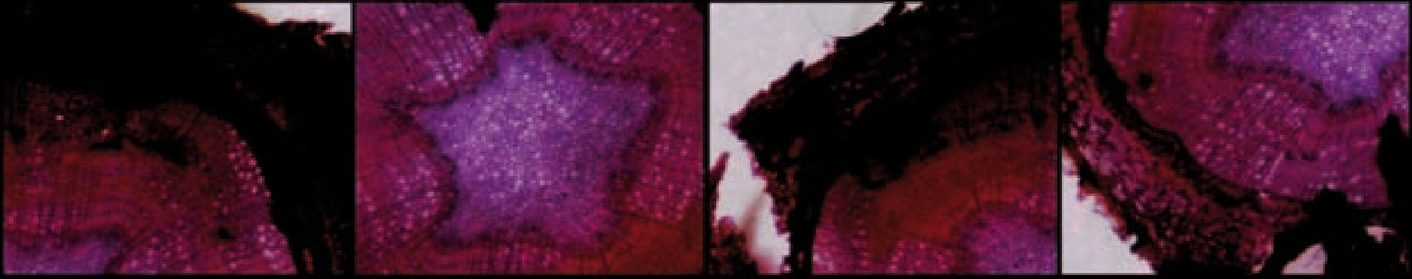

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Universitat Autònoma de Barcelona
Departament de Biologia Animal,
de Biologia Vegetal i d'Ecologia
Unitat d'Ecologia

DROUGHT-INDUCED DIE-OFF IN *PINUS SYLVESTRIS* AND *QUERCUS ILEX* FORESTS OF CATALONIA

Memoria presentada por:

Lucía Galiano Pérez

para optar al grado de Doctora

Con la aprobación de:

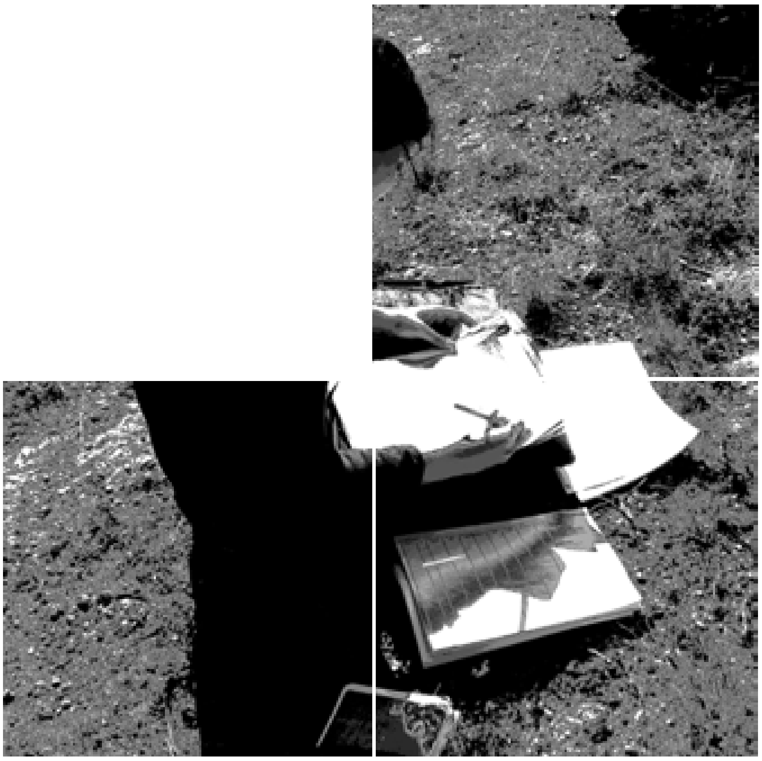
Dr. Francisco Lloret Maya	**Dr. Jordi Martínez-Vilalta**
Catedrático de Ecología	Profesor Agregado
Universitat Autònoma de Barcelona	Universitat Autònoma de Barcelona

Bellaterra, Marzo de 2012

A toda mi familia,
en especial a mis padres y hermana.
Y a vosotros, compañeros de camino.

“Un viaje de diez mil kilómetros empieza por un solo paso”

CONTENIDO



Capítulo 1

Introducción general

Mortalidad en bosques asociada al clima: causas y consecuencias

En los últimos años, se ha detectado un creciente número de episodios de mortalidad asociados a cambios en el clima en todo tipo de comunidades forestales del mundo (ver revisión en Allen *et al.* 2010). En particular, los eventos extremos de sequía someten a los árboles a un fuerte estrés fisiológico que, ocasionalmente, produce elevadas tasas de mortalidad y episodios de decaimiento forestal (Bréda *et al.* 2006). El decaimiento del bosque es un fenómeno que se caracteriza por una rápida defoliación de las copas y un aumento de la mortalidad (Bréda *et al.* 2006). Estos eventos de mortalidad tienen profundas implicaciones demográficas, y pueden alterar la vegetación de áreas extensas en periodos de tiempo relativamente cortos (McDowell *et al.* 2008). En consecuencia, en un futuro próximo cabe esperar que se produzcan modificaciones en la estructura y el funcionamiento de los ecosistemas forestales, en las interacciones que existen entre el bosque y el clima (Bonan 2008), así como en los bienes y servicios que éstos proporcionan a la sociedad (ver Figura 1.1). Además, estos eventos de mortalidad pueden interactuar con otro tipo de perturbaciones como son los incendios forestales, provocando importantes cambios en la dinámica de los ecosistemas terrestres a múltiples escalas (ver Allen 2007).

La muerte de un árbol es un proceso en el que pueden intervenir múltiples factores, tanto abióticos como bióticos, que frecuentemente interactúan de una manera compleja (Waring 1987; Franklin *et al.* 1987). Muchos estudios han asumido la hipótesis de Manion (1991) que considera tres fases para explicar el proceso de decaimiento del bosque: (1) los factores de predisposición son aquéllos que exponen al árbol a una situación de estrés a largo plazo, como son unas condiciones pobres de suelo y/o una elevada densidad de árboles; estos factores de predisposición hacen que los árboles sean más vulnerables a (2) factores de incitación que aceleran el declive del árbol a corto plazo, como son episodios extremos de sequía; y finalmente (3) los factores de contribución, generalmente patógenos oportunistas y/o episodios de sequía adicionales, que acaban matando el árbol previamente debilitado. A pesar de la robustez teórica de esta hipótesis, pocos estudios han analizado empíricamente la importancia relativa de todos los factores que potencialmente pueden estar implicados en los procesos de decaimiento inducidos por sequía.

Los patrones espaciales de decaimiento pueden dar pistas sobre sus posibles causas. Por ejemplo, patrones regionales de mortalidad apuntan hacia causas que actúan a grandes escalas determinadas por el clima. Por otro lado, patrones espaciales más localizados pueden indicar ataques puntuales de patógenos o apuntar hacia factores que modifican el control climático de la disponibilidad hídrica en el suelo a una escala local, como son la topografía y las propiedades del suelo (Western *et al.* 2002). En condiciones de sequía, cuando la evapotranspiración excede de manera continuada a la precipitación, las características topográficas como la altitud, la pendiente y la orientación, y las características del suelo, como la textura y la profundidad de los horizontes edáficos, modifican la disponibilidad hídrica a nivel local (Grayson *et al.*1997).

La presencia de agentes bióticos raramente se asocia a la muerte de un árbol si éste no está previamente debilitado. Sin embargo, las nuevas condiciones de cambio climático parecen estar potenciando las relaciones de retroalimentación positiva entre la dinámica poblacional de muchos parásitos y el debilitamiento fisiológico de las plantas huésped (Kurz *et al.* 2008; Boone *et al.* 2011). El aumento de las temperaturas puede modificar el ciclo reproductivo de muchos tipos de parásitos favoreciendo la aparición de brotes (Mattson & Haack 1987; Logan *et al.* 2003; Breshears *et al.* 2005). Al mismo tiempo, los cambios en la asignación de carbono inducidos por sequías severas o prolongadas pueden disminuir la producción de substancias de defensa (Waring 1987; Dunn *et al.* 1990; Croisé & Lieutier 1993), reduciendo la capacidad de los árboles a hacer frente, por ejemplo, al ataque de insectos xilófagos (Mattson & Haack 1987; Negrón *et al.* 2009). Las plantas hemiparásitas también pueden impactar en las relaciones hídricas de las plantas huésped como resultado de las grandes cantidades de agua que transpiran (Ehleringer & Marshall 1995). De hecho, numerosos estudios han documentado ya episodios de mortalidad en áreas muy extensas asociados a eventos de sequía extrema y al aumento de las poblaciones de parásitos (Williams & Liebhold 2002; Dobbertin *et al.* 2007; Negrón *et al.* 2009; Van Mantgem *et al.* 2009).

Mecanismos de mortalidad inducida por sequía a nivel fisiológico

El aumento en la intensidad y la frecuencia de las sequías que auguran los modelos de cambio climático (IPCC 2007) probablemente causará un incremento de los episodios de mortalidad forestal. Si queremos predecir estos efectos, necesitamos entender los factores que determinan la vulnerabilidad de las distintas especies a los cambios en las condiciones climáticas, y en particular, los mecanismos fisiológicos que subyacen a la muerte de un árbol en condiciones de sequía extrema. Aunque ya se habían propuesto antes en la literatura, McDowell *et al.* (2008) distinguieron formalmente tres mecanismos para explicar la mortalidad en árboles inducida por sequía: la fallida del sistema de transporte hidráulico, la inanición por agotamiento de las reservas de carbono, y el ataque de agentes bióticos (parásitos e insectos) que actúan agravando los efectos negativos de los dos mecanismos anteriores. El fallo hidráulico ocurre cuando las tensiones en el xilema en condiciones de estrés hídrico llevan a las plantas a niveles irreversibles de cavitación que interrumpen el flujo de savia y causan la pérdida de turgencia en las células (Tyree & Sperry 1989). La inanición por agotamiento de carbono se produciría porque el cierre estomático en condiciones de estrés provoca un desequilibrio entre la asimilación fotosintética y las demandas metabólicas (crecimiento y mantenimiento) de carbono. Si la sequía se prolonga en el tiempo, este balance negativo de carbono puede conducir al agotamiento de las reservas y la muerte del árbol por inanición (Parker & Patton 1975; Martínez-Vilalta *et al.* 2002; Bréda *et al.* 2006). Aunque ambos mecanismos resultan difíciles de demostrar en la naturaleza, existen evidencias convincentes en cuanto a la muerte parcial o completa de individuos por fallo hidráulico (Davis *et al.* 2002; Brodribb & Cochard 2009; Anderegg *et al.* 2012). Por el contrario, las evidencias a favor del mecanismo de muerte por inanición de carbono son mucho menos claras y esto ha generado recientemente un intenso debate (McDowell & Sevanto 2010; Sala *et al.* 2010; McDowell *et al.* 2011).

La regulación estomática de los flujos de savia en condiciones de estrés hídrico opera a lo largo de un continuo entre dos extremos (McDowell *et al.* 2008; Meinzer *et al.* 2009): (1) especies relativamente isohídricas, poco tolerantes al estrés hídrico, con una estricta regulación estomática para evitar potenciales hídricos bajos y consecuentes embolismos y cavitaciones del sistema vascular, y (2) especies relativamente anisohídricas con adaptaciones que les permiten tolerar potenciales hídricos muy bajos en sus tejidos y mantener, así, elevadas conductancias estomáticas y flujos de transpiración. McDowell *et al.* (2008) apuntaban a las especies isohídricas como aquéllas con un comportamiento más conservador en el uso de los recursos hídricos y, por lo tanto, con mayor probabilidad de morir por inanición de carbono en condiciones de sequía. Sin embargo, existen nuevas evidencias que demuestran que la cavitación ocurre en las especies isohídricas más frecuentemente de lo que se pensaba (Meinzer *et al.* 2009). Este hecho se explicaría, en primer lugar, porque los valores de potencial hídrico necesarios para el cierre estomático completo superan los umbrales de cavitación de muchas de estas especies. Además, se ha observado que estas especies experimentan ciclos diarios de cavitación y recarga de agua en los vasos conductores permitiendo mantener la transpiración en condiciones de estrés (ver también Hölttä *et al.* 2009a).

De acuerdo con lo expuesto hasta ahora, McDowell *et al.* (2011) han revisado su esquema inicial y proponen un nuevo marco hidráulico en el que los mecanismos de mortalidad ocurren de una manera más integrada como procesos interdependientes. Como ya se ha dicho anteriormente, el cierre estomático prolongado inducido por la escasez de agua en el suelo reduce las reservas de carbono como resultado del desequilibrio entre la asimilación fotosintética y las demandas metabólicas de carbono. Además de las demandas de carbono por respiración, el carbono es utilizado para llevar a cabo el ajuste osmótico necesario para el transporte en el floema (Münch 1930; Hölttä *et al.* 2009b), mantener la turgencia de las células (Brodribb & Holbrook 2006) y cubrir el gasto energético que implica rellenar de agua los vasos embolizados (Bucci *et al.* 2003; Salleo *et al.*2009). Esta interdependencia entre el transporte hidráulico y la disponibilidad de carbono puede llevar a un ciclo de retroalimentación en el que menos conductancia hidráulica implica menos asimilación fotosintética de carbono y más dependencia de las reservas de carbono para mantener la función osmótica. Al mismo tiempo, la baja disponibilidad de agua y carbono disminuye la capacidad defensiva a hacer frente al ataque de agentes bióticos (Dunn *et al.* 1990; Croisé & Lieutier 1993). Finalmente, la muerte podría ocurrir cuando uno o más de estos procesos alcanzaran un cierto umbral.

Los árboles maduros almacenan grandes cantidades de reservas de carbono móvil, principalmente compuestas por carbohidratos no estructurales (NSC, por sus siglas en inglés) y lípidos neutros (triacilglicéridos). Tradicionalmente, se ha considerado el tamaño de este pool de reservas como un indicador del estado de suministro de combustible que la planta tiene respecto al carbono, ya que debería reflejar cualquier disminución o acumulación en el pool de acuerdo con el balance entre la asimilación y el consumo de carbono (fotosíntesis versus metabolismo, crecimiento y exportación; Mooney 1972, Chapin *et al.* 1990; Körner 2003). De este modo, el carbono acumulado en un órgano o tejido puede ser posteriormente movilizado hacia tejidos que requieran carbono cuando estas demandas temporalmente excedan la asimilación (Chapin *et al.* 1990). Numerosos estudios experimentales han demostrado variaciones en el tamaño del pool de carbono en función del tipo de tejido (asimiladores versus consumidores) que se manipula. Por ejemplo se ha observado una disminución de reservas cuando los árboles son defoliados (Langström *et al.* 1990; Vanderklein & Reich 1999; Kosola *et al.* 2001; Li *et al.* 2002) y una acumulación cuando los árboles son desyemados (Chapin & Wardlaw 1988; Li *et al.* 2002). Sin embargo, Sala *et al.* (en revisión) aportan evidencias que sugieren que el almacenamiento de carbono podría ser un proceso activo. Como ya se ha dicho anteriormente, el carbono tiene un papel muy importante en la función osmótica, y particularmente en condiciones de estrés hídrico, de modo que las plantas podrían proveerse activamente de reservas que les permitieran, por ejemplo, mantener el transporte hidráulico del sistema conductor. En cualquier caso, puesto que existe una dinámica de removilización de reservas dentro de las plantas (Cherbuy *et al.* 2001), es importante hacer una medida integrada de las reservas de carbono teniendo en cuenta los diferentes compartimentos donde se puedan almacenar (hojas, ramas, tronco, raíces) (Hoch *et al.* 2003; Würth *et al.* 2005).

Las condiciones de sequía pueden limitar tanto las actividades asimiladoras (por cierre estomático) como las consumidoras de carbono (p. ej. crecimiento estructural). No obstante, se han observado respuestas dispares del tamaño del pool de reservas de carbono a las condiciones de estrés hídrico (estabilidad, incremento o disminución) que además parecen variar entre especies y fases ontogénicas (Sala *et al.* 2010). En este sentido, McDowell *et al.* (2011) ponen de relieve la importancia de tener en cuenta la intensidad y la duración de la sequía, puesto que en condiciones de sequía moderadas o en las primeras etapas de una sequía más extrema se puede observar un incremento transitorio en las reservas de carbono debido a que inicialmente la escasez de agua puede suponer un mayor impacto en los procesos de elongación de las células durante el crecimiento que en la fotosíntesis. Aunque existen algunas evidencias directas e indirectas consistentes con la hipótesis de muerte por inanición de carbono (Adams *et al.* 2009; Breshears *et al.* 2009), parece difícil descartar que otros mecanismos puedan estar interactuando (McDowell *et al.* 2011). Cabe decir que recientemente se han propuesto otros mecanismos como son la inmovilización de reservas o el fallo hidráulico en el floema que podrían llevar a la muerte de los árboles por inanición de carbono sin que necesariamente hubiera agotamiento absoluto de las reservas (Sala *et al.* 2010). Por lo tanto, son muchas las cuestiones que permanecen sin resolver en cuanto a los mecanismos que conducen a la mortalidad de árboles bajo condiciones de sequía extrema.

Cambios de vegetación a largo plazo: impacto de las sequías extremas sobre los patrones de reclutamiento

La dinámica de las comunidades forestales es el resultado del balance entre las tasas de crecimiento, mortalidad y reclutamiento de las poblaciones que las constituyen. La muerte de un árbol adulto proporciona un nuevo microambiente que favorece el inicio de una secuencia de sucesión que normalmente culmina con la substitución del dosel original por uno o más árboles nuevos (Hubbell & Foster 1986). No obstante, cuando la muerte de un árbol es inducida por sequía se generan unas condiciones particulares (ver Suarez & Kitzberger 2008): (1) la distinta vulnerabilidad a la sequía de las especies limita la producción y la viabilidad de las semillas de algunas especies en particular (Zlotin & Parmenter 2008; Mutke *et al.* 2005); (2) las condiciones relativamente sombrías que proporciona la presencia de los árboles muertos en pie y la consecuente ausencia de perturbación en los suelos tiende a beneficiar las plantas preexistentes en el sotobosque; (3) la ausencia de detritus de madera a corto plazo y los límites difusos de la apertura del dosel hacen que las condiciones sean más homogéneas en comparación a una apertura típica creada por la muerte y caída de los árboles; (4) en conjunto, las nuevas condiciones a nivel de la superficie del suelo son particularmente secas, ya que la falta de detritus disminuye la capacidad de retención del agua y, a su vez, el posible desarrollo de plantas preexistentes en el sotobosque incrementaría su consumo. Así, los episodios severos de mortalidad inducida por sequía que afectan unas especies en particular pueden no verse compensados por el reclutamiento de nuevas plántulas. Existen muy pocos estudios que analicen el impacto de las sequías extremas sobre las tendencias demográficas a largo plazo, especialmente trabajos que investiguen los patrones de reclutamiento (ver Van Mantgem & Stephenson 2007; Suarez & Kitzberger 2010). Se necesitan estudios específicos que determinen si los cambios ambientales en los nichos de regeneración asociados a mortalidad inducida por sequía favorecerán el reclutamiento de nuevas especies y/o el desarrollo de las especies preexistentes en el sotobosque. Estos procesos demográficos pueden conducir a cambios permanentes en la composición de especies de las comunidades forestales (ver Clinton *et al.* 1993; Slik 2004; Suarez & Kitzberger 2008).

El reclutamiento de plántulas es un proceso crítico para la regeneración debido a que las etapas tempranas del desarrollo de las plantas son normalmente más vulnerables que los adultos a las condiciones ambientales adversas (Cook 1979; Fenner 1987; Silvertown & Lovett-Doust 1993). El reclutamiento de las especies leñosas consiste en una secuencia de varias etapas conectadas por procesos de transición demográfica (dispersión, emergencia, supervivencia, crecimiento) (Grubb 1977; Harper 1977). Aunque son muchos los factores abióticos y bióticos que pueden interferir en estas etapas (llegada de semillas, luz, propiedades físicas y químicas del suelo, patógenos, herbívoros, competencia con hierbas) (Schupp 1993; Davis *et al.* 1999; Nicotra *et al.* 1999; Herrera 2002), la escasez de agua se ha considerado el principal factor limitante del establecimiento de plántulas en muchos ambientes (Moles & Westoby 2004; Moss & Hermanutz 2009). En particular, numerosos estudios han mostrado los efectos negativos que las condiciones de sequía (tanto experimentales como naturales) tienen sobre la dispersión y el banco de semillas, y sobre la emergencia, la supervivencia y el crecimiento de las plántulas (Borchert *et al.* 1989; Dunne & Parker 1999; Castro *et al.* 2004; Lloret *et al.* 2005; del Cacho *et al.* 2011). Esta vulnerabilidad a la escasez de agua que presentan numerosas especies y/o etapas demográficas podrían conducir a cambios permanentes en la estructura y composición de especies de las comunidades forestales si se cumplen las proyecciones de cambio climático que predicen un aumento de la aridez en numeroso ecosistemas terrestres (ver Engelbrecht & Kursar 2003; Engelbrecht *et al.* 2005; Marañón *et al.* 2004).

El caso de la cuenca mediterránea: especies estudiadas

En los climas mediterráneos, la disponibilidad hídrica representa el principal factor limitante para el crecimiento y la distribución de las especies de plantas (Mooney 1983; Terradas & Savé 1992). En particular, el clima de la cuenca mediterránea marca el límite sur del área de distribución de muchas especies de origen eurosiberiano, las cuales tenderían a ser más sensibles a los efectos del incremento en la aridez en los bordes meridionales - más secos - de sus distribuciones (Hampe & Petit 2005). Asimismo, los ecosistemas forestales de esta zona podrían verse especialmente afectados (Borghetti *et al.* 1998; Martínez-Vilalta & Piñol 2002; Ogaya *et al.* 2003; Sarris *et al.* 2007) si se cumplen las predicciones de cambio climático que auguran periodos de sequía más frecuentes e intensos en esta región (Christensen *et al.* 2007; IPCC 2007). En las últimas décadas, además de los efectos del cambio climático, las transformaciones en el uso humano del territorio han provocado la densificación y expansión de muchos bosques mediterráneos debido al abandono de la agricultura (Poyatos *et al.* 2003; Romero-Calcerrada & Perry 2004), a la aforestación artificial (Martínez-García 1999; Navarro *et al.* 2010) y al declive de la explotación forestal (Terradas 1999; Linares *et al.* 2009, 2010). Los bosques con una alta densidad de árboles normalmente experimentan una fuerte competencia por los recursos hídricos debido a la reducción en la disponibilidad de agua por árbol, lo cual se podría traducir en un agravamiento en la vulnerabilidad de los bosques a la escasez de agua (Bigler *et al.* 2006; Linares *et al.* 2009; Vilà-Cabrera *et al.* 2011).

Los ecosistemas mediterráneos muestran, al mismo tiempo, una alta resiliencia a las perturbaciones (Malanson & Trabaud 1987). Muchos árboles y arbustos presentan una apreciable capacidad de rebrotar tras sufrir una perturbación (Canadell & Zedler 1995), lo cual constituye un elemento clave para la resiliencia de estas comunidades (*sensu* Westman 1986). Muchas especies mediterráneas leñosas poseen un gran lignotuber – un hinchamiento lignificado en la base del tronco (James 1984) –, que actúa como reservorio de yemas durmientes, carbohidratos y nutrientes de reserva que permiten la rápida regeneración después de una perturbación (Mullette & Bamber 1978; Canadell & Zedler 1995). No obstante, perturbaciones severas pueden producir la pérdida de resiliencia debido al agotamiento de las reservas de los órganos supervivientes, lo cual puede ocurrir particularmente si una nueva perturbación tiene lugar antes de que los niveles de reservas se hayan recuperado (Jones & Laude 1960; Rundel *et al.* 1987; Malanson & Trabaud 1988). Algunos estudios muestran como las perturbaciones recurrentes resultan en la progresiva pérdida de resiliencia de los ecosistemas mediterráneos (Díaz-Delgado *et al.* 2002; Lloret *et al.* 2004). Aunque varios estudios han examinado cómo la capacidad de resiliencia depende de las tasas de reposición de las reservas de carbono tras perturbaciones inducidas experimentalmente (p. ej. tala; Canadell & López-Soria 1998; López *et al.* 2009), todavía se sabe muy poco a cerca de la respuesta de las reservas frente a episodios de sequía en condiciones naturales.

El pino albar (*Pinus sylvestris* L.) es una de las especies de árbol más ampliamente distribuidas de la Tierra. La alta plasticidad que presenta en cuanto a su arquitectura hidráulica (Poyatos *et al.* 2007; Martínez-Vilalta *et al.* 2009), junto con su estricto control de cierre estomático en condiciones de estrés hídrico (Irvine *et al.* 1998), parecen ser las causas que le permiten ocupar un extenso gradiente climático que va desde las regiones boreales, donde se hallan las mayores poblaciones, hasta regiones más secas en la cuenca mediterránea (Barbéro *et al.* 1998). El límite sud–oeste del área de distribución del pino albar se sitúa en la Península Ibérica, donde se encuentran poblaciones importantes en los Pirineos y algunas poblaciones dispersas en localidades más meridionales y secas (Castroviejo *et al.* 1986). Existen claros indicios de que en esta región las poblaciones de pino albar estan sufriendo recientes episodios de mortalidad asociados a sequía, (Martínez-Vilalta & Piñol 2002; Hódar *et al.* 2003), de forma parecida a lo que se observa en los valles más secos de los Alpes suizos (Bigler *et al.* 2006).

La encina (*Quercus ilex* L.), a su vez, es un clásico ejemplo de especie mediterránea que es capaz de rebrotar desde el lignotuber después de una perturbación. Aunque las mayores poblaciones de esta especie se hallan en el oeste de la cuenca mediterránea (Barbéro *et al.* 1992), la encina está presente en una amplia área que se extiende desde Portugal a Siria, y desde Marruecos a Algeria y Francia (Terradas 1999). La encina ha sido tradicionalmente considerada como una típica especie mediterránea resistente a la sequía (Canadell & Rodà 1991; Savé *et al.* 1999; Infante *et al.* 2003). Sin embargo, muchos estudios muestran como, en condiciones extremas de estrés hídrico, la encina puede experimentar potenciales hídricos relativamente bajos en las hojas (Limousin *et al.* 2009), elevadas pérdidas de conductividad hidráulica en el xilema (Tognetti *et al.* 1998; Martínez-Vilalta *et al.* 2003) y balances de carbono negativos (Gracia *et al*. 2001). De hecho, ya se han documentado defoliaciones masivas de encinas asociadas con sequía en la cuenca mediterránea, lo que indica que esta especie sería más vulnerable a la escasez de agua que otras especies concurrentes (Lloret & Siscart 1995; Peñuelas *et al.* 2000; Peñuelas *et al.* 2001; Martínez-Vilalta *et al.* 2002; Lorenz *et al.* 2005).

Objetivos y estructura de la tesis

Esta tesis profundiza en el conocimiento de las causas y las consecuencias de la mortalidad en bosques asociada al clima. El objetivo general se centra en analizar cuáles son los principales factores que determinan la supervivencia de los árboles en las condiciones que se producen en episodios de sequía extrema, así como evaluar aquellos factores que contribuyen a la posterior recuperación. El enfoque de la investigación implica diferentes escalas, desde el nivel fisiológico que considera los mecanismos que subyacen a la muerte de un árbol hasta las consecuencias que estas mortalidades tienen a nivel de comunidad (Figura 1.1). Para ello, se han analizado dos episodios recientes de mortalidad inducida por sequía en dos localidades apartadas en el Pirineo catalán. Uno tuvo lugar durante los años 2004-2005 y afectó una población de pino albar (*Pinus sylvestris* L.), una especie sin capacidad de rebrotar después de una perturbación y con el límite meridional de su área de distribución en la Península Ibérica. El otro episodio tuvo lugar durante el verano del año 2000 y afectó a una población de encina (*Quercus ilex* L.), una especie típicamente mediterránea y con una elevada capacidad de rebrote tras sufrir una perturbación.

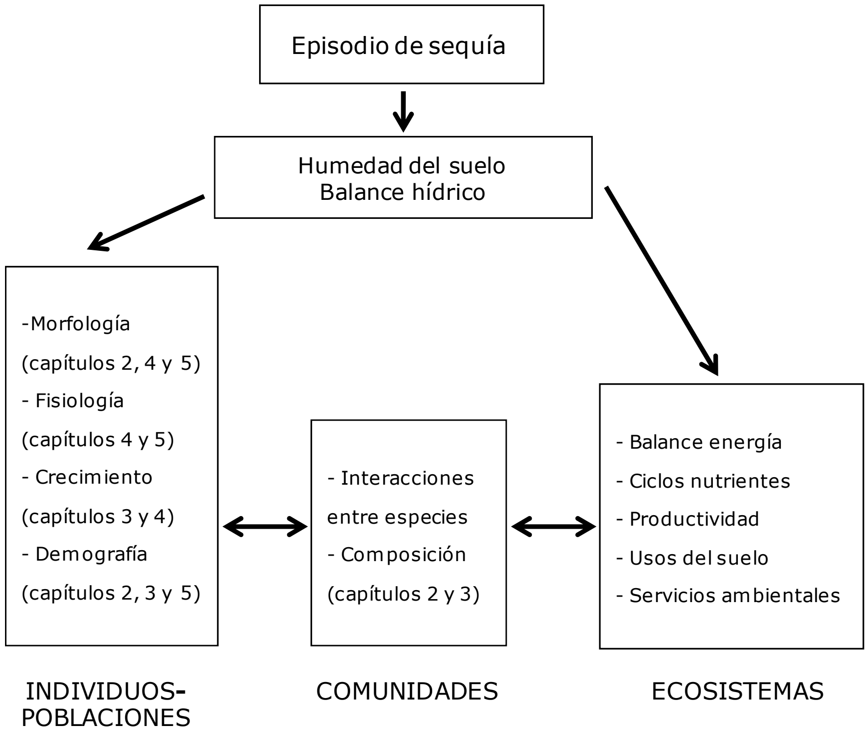


Figura 1.1. Efectos de la sequía y escalas a las que se producen. Los procesos entre escalas y dentro de escalas pueden interaccionar entre ellos. Se indican los capítulos donde están tratados cada uno de los procesos.

Además de este primer capítulo introductorio y de un último capítulo de discusión y conclusiones generales, esta tesis consta de cuatro capítulos en los que se han investigado los siguientes objetivos específicos:

CAPÍTULO 2: **Drought-induced multifactor decline of *Pinus sylvestris* in the Pyrenees and potential vegetation change by the expansion of co-occurring oak species.**

En este capítulo se examina el amplio rango de factores que estarían asociados a los patrones observados de defoliación y mortalidad del pino albar en respuesta a un episodio reciente de sequía extrema, haciendo especial énfasis en el papel de factores intrínsecos del bosque como la estructura de la población de pinos y las propiedades del suelo, y de factores extrínsecos relacionados con la interacción con otras especies como la infección por muérdago. Se analizan también los patrones de reclutamiento de las principales especies leñosas presentes en la comunidad.

CAPÍTULO 3: **Seedling emergence and growth of *Quercus* spp. following severe drought effects on a *Pinus sylvestris* canopy.**

En este capítulo se estudian los patrones de reclutamiento de *Quercus ilex* L. y *Quercus humilis* Mill. en relación a los efectos del episodio de sequía en las copas dominantes de pino albar. Dada su área de distribución actual en la región mediterránea, en un contexto de cambio climático estas dos especies de *Quercus* podrían reemplazar al pino albar. Concretamente, se analiza si los cambios ambientales en los nichos de regeneración asociados a la mortalidad y la defoliación del pino albar inducidos por el episodio de sequía favorecen las fases de emergencia y/o el crecimiento de las plántulas de *Quercus* del sotobosque, lo cual podría conducir al bosque a un cambio en la composición de especies.

CAPÍTULO 4: **Carbon reserves and canopy defoliation determine the recovery of *Pinus sylvestris* four years after a drought episode.**

En este capítulo se evalúa el estado de recuperación del bosque de pino albar cuatro años después del episodio de sequía en relación a diferentes factores como la disminución del crecimiento durante el episodio o la infección por muérdago. En particular, se analizan los niveles de reservas de carbono y la defoliación de las copas, considerando que son factores claves en la recuperación y la supervivencia de los árboles.

CAPÍTULO 5**: Determinants of prolonged drought effects on crown condition and their relationship with depletion of carbon reserves in a Mediterranean holm oak forest.**

En este capítulo se estudian los efectos prolongados en el tiempo de una sequía extrema en una población de encinas siete años después del evento. Concretamente, se examinan los factores que están asociados a los patrones de afectación de la copa, haciendo especial énfasis en el papel de factores intrínsecos del bosque como la estructura de la población de encinas y las propiedades del suelo. Adicionalmente, se evalúa la relación entre la condición de las copas y las reservas de carbono y nutrientes almacenadas en el lignotuber siete años después del episodio de sequía.

Los cuatro capítulos centrales que constituyen propiamente la investigación original de esta tesis están redactados en formato de artículo científico, por lo que alguna información puede aparecer de forma redundante. La lengua utilizada en el redactado de estos capítulos es el inglés, aunque los resúmenes están también traducidos al castellano. Algunos capítulos ya están enviados o publicados en revistas científicas de carácter internacional, como se indica en la portada de cada capítulo. La bibliografía se ha unificado al final de la tesis para evitar repeticiones.

Capítulo 2

Drought-induced multifactor decline of *Pinus sylvestris* in the Pyrenees and potential vegetation change by the expansion of co-occurring oak species

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Galiano L, Martínez-Vilalta J and Lloret F, 2010

RESUMEN

Declive multifactorial inducido por sequía de *Pinus sylvestris* en el Pirineo y potencial cambio de vegetación por la expansión de quercíneas coexistentes

Recientemente, se han observado episodios de mortalidad inducida por sequía en muchos bosques del mundo, particularmente en los límites más áridos del área de distribución de las especies. En el contexto del cambio climático, estos datos apuntan hacia potenciales cambios de vegetación que podrían ocurrir en extensas áreas geográficas, con un gran impacto en la estructura y el funcionamiento de los ecosistemas. En este capítulo se estudian los efectos de un episodio de sequía que tuvo lugar en los años 2004-2005 en una población de *Pinus sylvestris* L. en los Pirineos centrales (NE España). Nuestro principal objetivo fue analizar las correlaciones ambientales con el declive del bosque y el cambio de vegetación observado a nivel de parcela. Se utilizaron modelos lineales generales y generalizados para estudiar la relación entre la defoliación del dosel, la mortalidad de árboles y el reclutamiento de nuevos individuos, y las características de las parcelas. Los resultados mostraron un declive asociado a múltiples factores desencadenado por la sequía del 2004-2005. Tanto la defoliación como la mortalidad se relacionaron con el nivel de sequía estimado para cada parcela. Adicionalmente, la estructura del bosque, las propiedades del suelo y la infección por muérdago también se relacionaron con los patrones de defoliación observados, probablemente actuando como factores de predisposición a largo plazo. El reclutamiento de *P. sylvestris* fue bajo. Por el contrario, observamos un abundante establecimiento de otras especies arbóreas, como *Quercus ilex* L. y *Q. humilis* Mill., particularmente en aquellas parcelas donde *P. sylvestris* mostraba elevadas tasas de defoliación y mortalidad. Estos resultados sugieren una migración de quercíneas hacia altitudes superiores en la ladera mediada por el declive de la especie dominante. En consecuencia, si se cumplen los escenarios de cambio climático muchas poblaciones de *P. sylvestris* que se encuentran refugiadas en ambientes montanos en la Península Ibérica podrían estar en riesgo en un futuro.

ABSTRACT

Episodes of drought-induced tree dieback have been recently observed in many forest areas of the world, particularly at the dry edge of species distributions. Under climate change, those effects could signal potential vegetation shifts occurring over large geographical areas, with major impacts on ecosystem form and function. In this article, we studied the effect of a single drought episode, which occurred in years 2004-2005, on a *Pinus sylvestris* L. population in central Pyrenees (NE Spain). Our main objective was to study the environmental correlates of forest decline and vegetation change at the plot level. General and generalized linear models were used to study the relationship between canopy defoliation, mortality and recruitment, and plot characteristics. A drought-driven multifactor dieback was observed in the study forest. Defoliation and mortality were associated with the local level of drought stress estimated at each plot. In addition, stand structure, soil properties and mistletoe infection were also associated with the observed pattern of defoliation, presumably acting as long-term predisposing factors. Recruitment of *P. sylvestris* was low in all plots. In contrast, we observed abundant recruitment of other tree species, mostly *Quercus ilex* L. and *Q. humilis* Mill., particularly in plots where *P. sylvestris* showed high defoliation and mortality. These results suggest that an altitudinal upwards migration of *Quercus* species, mediated by the dieback of the currently dominant species, may take place in the studied slopes. Many rear-edge populations of *P. sylvestris* sheltered in the mountain environments of the Iberian Peninsula could be at risk under future climate scenarios.

INTRODUCTION

Climate-related forest dieback is a recurrent phenomenon that has already been reported in a variety of woodland and forest communities in many parts of the world (Auclair 1993; Williamson *et al.* 2000; Rice *et al.* 2004; Allen *et al.* 2010; Van Mantgem *et al.* 2009). Climate change may increase physiological stress on long-lived woody vegetation, occasionally leading to increased mortality rates and episodes of forest dieback (Allen *et al.* 2010). This dieback is characterized by rapid defoliation and progressive increase in mortality of overstory trees (Bréda *et al.* 2006). Such widespread mortality events have the capacity to transform regional landscapes on a sub-decadal timescale, with significant implications for stand structure and dynamics and ecosystem function (McDowell *et al.* 2008).

Our current understanding of the mechanisms underlying tree dieback is still limited (Bréda *et al.* 2006; McDowell *et al.* 2008). Tree death is a complex process that has been attributed to a wide range of potential causes, often involving prior droughts that initiate a growth decline (Pedersen 1998; Oberhuber 2001; Camarero *et al.* 2002; Das *et al.* 2007) and a lengthy chain of interacting events (Waring 1987; Franklin *et al.* 1987). Thus, many studies have assumed a slow-decline hypothesis (Manion 1991) that considers a three-stage decline: (1) predisposing factors expose plants to long-term stress, for instance, poor soil conditions and/or high stand density; these predisposing factors increase the susceptibility of trees to (2) severe short-term stresses known as inciting factors, for instance an extreme drought; and (3) eventually the contributing factors (for instance parasites and/or additional climatic events) can kill the trees. Despite the robust theoretical background of this hypothesis, the empirical support is relatively weak because the studies documenting drought-induced dieback rarely analyze the relative importance of all the factors that are potentially involved.

The large-scale control of climate on water availability in the soil may also be influenced by topography and soil properties at the local scale (Stephenson 1990; Western *et al.* 2002). In Mediterranean regions, especially in summer, when evapotranspiration continuously exceeds precipitation, topographic characteristics such as altitude, slope, aspect, and texture and depth of soil influence soil water availability as local controls (Grayson *et al.* 1997). Despite the importance of these factors on the spatial patterns of tree mortality, few studies have evaluated their effects, with the exception of the effect of altitude (Allen & Breshears 1998; Guarín & Taylor 2005).

Parasite outbreaks may be enhanced by climate change (Mattson & Haack 1987; Breshears *et al.* 2005). In addition to modifications in the parasite life cycle, climate-induced changes in plant carbon allocation may diminish the production of defence compounds (Waring 1987; Dunn *et al.* 1990; Croisé & Lieutier 1993), reducing the ability of trees to withstand, for instance, the attack of cambium-eating insects (Mattson & Haack 1987; Negrón *et al.* 2009). Hemiparasitic plants may also impact on host-water relations as a result of their high rates of transpiration, needed to take up water, nutrients and carbohydrates from host trees (Ehleringer & Marshall 1995). Therefore, increased vulnerability of trees to parasite attacks under water stress conditions can lead to episodes of high tree mortality (Williams & Liebhold 2002; Breshears *et al.* 2005; Dobbertin *et al.* 2007; Negrón *et al.* 2009).

In recent decades, changes in human use of many temperate forests have resulted in denser stands due to abandonment of agropastoral activities (Barbéro *et al.* 1998; Poyatos *et al.* 2003), to artificial afforestation (Martínez-García 1999) and to decreases in logging practices (Linares *et al.* 2009). Denser stands normally result in stronger plant competition (Linares *et al.* 2009) and "natural" self-thinning processes (Peet & Christensen 1987; Kenkel 1988; Chen *et al.* 2008), because of the corresponding reduction in soil water availability per unit of basal area. Therefore, trees in dense stands may be more predisposed to die under water stress conditions (Guarín & Taylor 2005; Bigler *et al.* 2006).

Forest communities are dynamic and changes occur continuously at the individual and population levels, due to a balance between growth, recruitment and mortality. Light-gaps are created when trees die in closed-canopy forests, initiating a micro-successional sequence that culminates in the replacement of the original canopy tree by one or more new trees (Hubbell & Foster 1986). Because altered conditions of light and soil moisture are expected in the gaps compared with conditions under the canopy (Suarez & Kitzberger 2008), recruitment of new species with different resource requirements could occur, with major implications for community dynamics (Connell 1978). Consistent with this, changes in the recruitment pattern associated to high mortality rates may promote shifts in species composition and in distribution areas in response to drought episodes. Nevertheless, research on the changes in recruitment patterns associated to drought-induced mortality in the overstory vegetation is still scarce (but see Condit 1998; Slik 2004; Kelly & Goulden 2008).

Scots pine (*Pinus sylvestris* L.) is one of the most widely distributed trees on Earth. Although the largest populations of this species occur in boreal regions, *P. sylvestris* also occupies large areas in relatively dry regions within the Mediterranean basin, from the Iberian Peninsula to Turkey (Barbéro *et al.* 1998). The south-western limit of *P. sylvestris* is in the Iberian Peninsula, with important populations in the Pyrenees and several populations scattered in southern, more arid localities (Castroviejo *et al.* 1986). These populations, located at the low-latitude, rear-edge of the species distribution area, are likely to be particularly sensitive to the effects of increased aridity (Hampe & Petit 2005). In fact, drought-related *P. sylvestris* dieback has already been reported in the Iberian Peninsula (Martínez-Vilalta & Piñol 2002; Hódar *et al.* 2003) and in the Alps (Rebetez & Dobbertin 2004; Bigler *et al.* 2006). As the Mediterranean basin climate is becoming warmer and drier (IPCC 2007), an increase in drought-induced mortality of *P. sylvestris* has been predicted (Martínez-Vilalta & Piñol 2002).

The main objective of this study is to evaluate drought as the potential inciting factor of the dieback observed in southern *P. sylvestris* forests. Although several studies have analyzed drought-related dieback episodes in many regions of the world (Rice *et al.* 2004; Mueller *et al.* 2005; Bigler *et al.* 2006; Van Mantgem & Stephenson 2007), the many factors potentially associated with forest decline have been rarely examined exhaustively. We emphasize here the role of the forest intrinsic factors such as stand structure and soil properties, and extrinsic factors such as mistletoe infection. We study the stand demographic trends in response to a recent drought episode, accounting for the patterns of defoliation and mortality, as well as recruitment at the level of a single valley in the Central Pyrenees (NE Spain). Three main questions are addressed: a) Are mortality and defoliation associated with the local level of drought stress estimated for each plot? b) Does the effect of drought stress depend on other factors such as soil properties or stand-level competition? c) Is the recruitment of other species related to the drought effects on the *P. sylvestris* tree canopy?

MATERIALS AND METHODS

Experimental site

The study was carried out in a *Pinus sylvestris* forest located in Central Pyrenees (Soriguera, Pallars Sobirà, 42º 22´ 43´´N, 1º 6´ 29´´E, ca. 16 km^2). Most of the *P. sylvestris* forests in the area have traditionally been under important agropastoral pressures up until the early twentieth century, and some forest patches were completely removed. Regarding the management practices at the study stands, selective activities on species establishment have never taken place in the area and logging activities on *P. sylvestris* stands have not been practised since the 1980s (Carles Fañanàs, Catalan Forest Service, pers. com.). Although both natural and artificial afforestation have been taking place in the area during the twentieth century, the study site appears absolutely within the distribution area of *P. sylvestris* and this species has naturally been present in the area since the Catalan Forest Service began keeping records. Accordingly, the studied *P. sylvestris* population exhibits natural regeneration and an uneven age and size structure (Appendix 2.1). This is further corroborated by dendrochronological analyses of tree-rings showing that *P. sylvestris* with a diameter between 20 and 40 cm have ages ranging between 30 and 100 years (Hereş *et al.* 2011). These forests are mainly on northern slopes and are continuously distributed in altitudes from 600 to 1500 m a.s.l. The shrub layer is predominantly occupied by *Buxus sempervirens* L., *Amelanchier ovalis* Medik. and *Lonicera xylosteum* L. Other species of trees, *Quercus humilis* Mill., *Quercus ilex* L. and *Betula pendula* Roth., occasionally appear in the understory, mostly at lower (*Quercus* species) and higher altitudes, respectively. Some younger *Pinus nigra* Arnold. plantations interrupt the *P. sylvestris* forest. A well-established mistletoe population, with individuals up to 30 years old, infects most *P. sylvestris* trees in the area.

The climate of the region is characterized by an annual mean temperature of 9.6 ºC and an annual mean rainfall around 643 mm (climate data obtained from the Spanish meteorological station network for the period 1951-1999, AEMET, Agencia Estatal de Meteorología), corresponding to the temperate oceanic submediterranean bioclimatic region (Worldwide Bioclimatic Classification System 1996-2009). In 2004-2005, the Iberian Peninsula experienced a severe drought episode preceded by several dry periods during the last years (EEA 2008). This drought affected our study area (Appendix 2.2), and was associated with the observation of evident damage on *P. sylvestris* stands (Carles Fañanàs, Catalan Forest Service, pers. com.). This observation was supported by dendroecological analyses that showed lower growth and higher mortality in 2004-2005 and subsequent years, although minor mortality episodes were also detected prior to these years (Hereş *et al.* 2011).

Field sampling methods

In August 2007, 30 circular plots (314 m^2 each) were sampled along a steep altitudinal gradient from 645 to 1383 m a.s.l. in two nearby slopes within the same valley. To avoid excessive environmental heterogeneity, plot selection used the following criteria: (a) slopes from 10º to 40º and North aspect; (b) no signs of recent disturbance or management; (c) distance between plots greater than 50 m.

Plots were defined around a central *P. sylvestris* tree. In each plot, trees, shrubs, and the presence of parasites, that is, the epiphyte mistletoe (*Viscum album* L.), were recorded (see below). All trees and shrubs were identified to species. Two size classes were considered for all woody species including *P. sylvestris*:

- Seedlings (< 50 cm height and < 1.5 cm diameter at breast height (DBH)) were recorded at the least disturbed 1 m width side of the 20 m central transect of the plot that was perpendicular to the slope. Seedling sprouts and seedlings may look quite similar when their height is close to 50 cm. We felt the root collar zone to detect possible important lignifications which would have evidenced that sprouts emerged from the roots.

- Adult trees (> 50 cm height and > 1.5 cm DBH) were recorded all over the plot. DBH was measured on all adult trees.

For all adult *P. sylvestris* individuals, additional information was recorded: state (dead or alive), visual estimation of the percentage of crown defoliation (%), presence/absence of bark beetles, and mistletoe occurrence (0 = no mistletoe, 1 = low mistletoe infestation with few mistletoe plants, 2 = medium mistletoe infestation with several large mistletoe bushes or many small plants found in the crown, 3 = heavy mistletoe infestation with many large mistletoes on at least one-third of the branches in the tree crown; Dobbertin & Rigling 2006). In each plot, a mistletoe index was calculated by averaging mistletoe occurrence across trees.

Response variables

Canopy defoliation and standing mortality were the response variables used to assess forest decline in this study. At the plot level, canopy defoliation (%) was calculated by averaging the percentage of crown defoliation for all living individuals, weighted by the basal area of each tree. Total canopy defoliation including dead trees was also calculated and used as predictor variable in the recruitment analysis (see below). Mortality was measured as the count of dead individuals relative to the total number of individuals (see "Statistical analyses" section). Patterns of recruitment for *Quercus ilex* and *Q. humilis* were also studied at the plot level, on the basis that these species could potentially replace *P. sylvestris* under future climatic conditions. Seedling abundance of *Q. ilex* and *Q. humilis* were pooled together because of their relatively similar characteristics and abundance pattern along the altitudinal gradient (see Figure 2.4a).

Forest structural variables and competition

Forest structural variables were recorded on the basis that competition could be affecting *P. sylvestris* defoliation and mortality patterns. At the plot level, intra-specific (*P. sylvestris*) competition was assessed using stand density, mean DBH, and an aggregation index (z value) based on distances to the nearest neighbour recorded for all *P. sylvestris* trees (Clark & Evans 1954). Large values of this index ($z > 1.96$) imply a regular distribution of trees and small values ($z < -1.96$) imply a clumped pattern, whereas values of z greater than -1.96 or less than 1.96 indicate a random distribution. At the individual level, we computed the Hegyi index of competition (Hegyi 1974) in a 5-m radius around each individual. This index takes into account the distance of the competitor to the focal tree as well as the size of the trees. Competition indexes were corrected for edge effects by assuming that the distribution of individuals inside and outside plots was similar.

Water availability

Water availability in the soil is mainly determined by soil properties at small scales and by the balance between net rainfall and evapotranspiration as well as topography at larger spatial scales (Western *et al.* 2002). Three sets of variables were thus used to characterize local water availability at the plot level. Note that these variables are not a precise record of conditions occurring during the 2004-2005 drought episode and should be considered only as relative indicators of water availability among plots.

a) *Summer water availability index*

Summer water availability (SWA) was assessed as: SWA = P / PET, where P is the average (1951-1999) precipitation from June to August and PET is the average potential evapotranspiration for the same period. Given that there was no weather station within the sampled altitude gradient, the monthly values of temperature and precipitation, as well as their altitudinal lapse rates, were obtained from the Climatic Digital Atlas of Catalonia (CDAC) (Pons 1996; Ninyerola *et al.* 2000), at a resolution of 180 m. The Hargreaves equation (Hargreaves & Samani 1985) was used to compute PET, accounting for the extraterrestrial solar radiation (Allen *et al.* 1998). Summer water availability ranged from 0.44 to 0.66.

b) *Topographic wetness index*

Topographic wetness indices developed by different authors (Beven & Kirkby 1979; O'Loughlin 1986) have long been used to characterize spatial soil moisture conditions at within-catchment scales. They assume that, from a simplified but realistic physical approach, topography is dominant in controlling and modifying the hydrologic processes operating at the hillslope scale (Grayson *et al.* 1999). We used the topographic index developed by Beven & Kirkby (1979), which accounts for the contributing area in the catchment that drains into a given point, and for the slope of the terrain. As the plots were distributed only on northern slopes, the aspect was not relevant in our case. The topographic index was computed from a Digital Elevation Model (DEM) with a resolution of 30 m, together with topographic information recorded at the plots. Topographic wetness index ranged from 7.62 to 11.60, with higher values representing wetter conditions.

c) *Soil texture measurements*

The studied soils are calcareous and belong predominantly to the clayey-loam texture class. We excavated one profile per plot and extracted a soil sample from 20 cm depth using a metal core (height = 7 cm, diameter = 5.6 cm) to determine the bulk density and the soil texture. The measurements were performed in the laboratory using mechanical methods. Soils were separated into the following size fractions (%): coarse sand (2-0.2 mm], fine sand (0.2-0.02 mm], silt (0.02-0.002 mm] and clay (< 0.002 mm). We computed the *B* coefficient from the Saxton equation (Saxton *et al.* 1986) with the purpose of obtaining a representative value of the water retention capacity from the texture of the soil. Large values of this index are characteristic of sandy soils, and imply a low water retention capacity. The fact that the studied soils were fairly rocky (44 % of large stones on overage) made difficult to excavate the C horizon. Thus, we used the depth of the upper (A and B) horizons as a measure of soil depth.

Statistical analyses

Statistical analyses were conducted at the individual tree and at the plot levels. At the plot level, most variables were normally distributed or normalizable using standard transformations (see Table 2.1). Stand density, dead individuals (%) and seedling abundance of *Quercus* species, however, remained non-normal. General and generalized linear models were thus used to study the relationship between plot characteristics and defoliation, mortality and recruitment. For the defoliation model, an identity link function was used because the canopy defoliation variable was normally distributed. In the mortality model, the number of standing dead *P. sylvestris* trees in each plot was considered a count response variable; the logarithm of the total number of *P. sylvestris* trees was introduced into the model as an explanatory variable. We used this approach because this response variable was not normalizable after standard transformations and it is effectively very similar to modelling the proportion of dead individuals (Faraway 2006). For the mortality and recruitment models, preliminary analyses using a Poisson distribution of errors for count variables showed overdispersion, and thus a negative binomial distribution was used in the final models (White & Bennetts 1996). All models (defoliation, mortality and recruitment) were as similar as possible in terms of explanatory variables (see Table 2.1). The occurrence of bark beetles was not introduced in the models because bark beetles appeared basically on dead trees, and thus were likely a consequence, not a cause, of tree mortality.

At the individual level, general and generalized mixed linear models were used to study the relationship between individual characteristics and defoliation (normally distributed) and mortality (binomially distributed). Among the explanatory variables, solely the Hegyi competition index was normalizable using standard transformations (see Table 2.1). DBH and mistletoe occurrence remained non-normal. Mistletoe occurrence was observed essentially on living trees and was tested as a fixed factor with four abundance classes in the defoliation model. Plot effects were modelled as a random factor in both individual-level models in order to account for the spatial autocorrelation among individuals within a plot.

Parameters (β) of all fitted models were estimated using maximum likelihood methods. Model selection was based on a stepwise selection procedure using the Aikake information criterion (AIC). Some additional analyses were conducted using Pearson and Spearman correlation coefficients as a measure of association between pairs of variables. Analysis of variance (One-Way ANOVA using Post Hoc Multiple Comparisons) was used to compare means between more than two groups. All statistical analyses were carried out with R version 2.9.0. (2009 The R Foundation for Statistical Computing).

Table 2.1. Explanatory variables introduced into the defoliation, mortality and recruitment models at the individual and plot levels (see text for details).

	Description of the explanatory variables	Units	Model
Plot-level (N = 30)	Summer water availability index (arcsin transformation)	adimensional	All
	Stand density	individuals/m^2	All
	Mean DBH	cm	All
	Aggregation index	adimensional	All
	Topographic wetness index	adimensional	All
	Saxton *B* coefficient (inverse transformation)	%	All
	Depth of the soil	cm	All
	Mistletoe index (log transformation)	adimensional	Defoliation and mortality
	Weighted percentage of total canopy defoliation including dead trees	%	Recruitment
	Total individuals (log transformation)	individuals/plot	Mortality
Individual-level (N = 1002)	DBH	cm	All
	Hegyi competition index (log transformation)	adimensional	All
	Mistletoe occurrence	adimensional	All

RESULTS

Determinants of defoliation and mortality patterns

At the plot level, average defoliation and standing mortality of *Pinus sylvestris* were 45.18% and 14.3%, respectively (Table 2.2). In contrast, no mortality was found in *Quercus* species. The final models fitted using stepwise model selection from the same initial set of explanatory variables differed notably for defoliation and mortality (Table 2.3). Both defoliation and mortality were mostly affected by summer water availability and stand density, but canopy defoliation was associated with additional explanatory variables (Table 2.3). Increases in defoliation and mortality were associated with lower summer water availability and higher stand density (as shown by the $\beta > 1$ for the total number of individuals in the mortality model). Indeed, defoliation and mortality of *P. sylvestris* tended to be higher at low and intermediate altitudes coinciding with lower climatic water availability (Figure 2.1). Mean DBH and, to a lesser extent, mistletoe index were also significant in the defoliation model; larger values of those variables were associated with higher canopy defoliation. In contrast, soil properties such as the Saxton *B* coefficient and soil depth were negatively correlated with canopy defoliation, although the relationship was only marginally significant in the case of soil depth. Interactions among explanatory variables were not significant for either defoliation or mortality. There was a positive relationship between canopy defoliation and mortality ($r = 0.620$, $P < 0.001$) indicating that the most affected plots in terms of mortality were still highly defoliated two years after the drought event.

Table 2.2. Structural attributes of the studied stands. Standard errors are in brackets.

	Living individuals	Dead individuals	Total stand
Number of individuals	856	146	1002
Mean dbh (cm)	18.24 (0.36)	11.95 (0.84)	17.32 (0.34)
Stand density (individuals/ha)	…	…	1071 (81)
Basal area (m^2/ha)	…	…	35.90 (2.7)
Canopy defoliation (weighted %)	…	…	45.18 (2.89)
Canopy defoliation (%)	…	…	51.05 (2.65)
Dead individuals (%)	…	…	14.3 (2.65)
Dead basal area (%)	…	…	9.13 (2.32)

Table 2.3. General and generalized linear models for defoliation and mortality, respectively, at the plot level. A stepwise model selection was used in both cases starting from the same initial set of explanatory variables. Only the final models are shown. N = 30 plots; $\text{AIC}_{\text{defoliation model}} = 142.62$; $\text{AIC}_{\text{mortality model}} = 155.20$.

	Model terms	β	SE	P
Defoliation plot-level model	Summer water availability index	-133.569	27.850	<0.001
	Stand density	252.394	50.963	<0.001
	Mean DBH	1.438	0.408	0.002
	Mistletoe index	8.952	3.645	0.022
	Saxton *B* coefficient	-298.505	122.384	0.023
	Soil depth	-0.250	0.125	0.057
Mortality plot-level model	Summer water availability index	-6.251	2.073	0.003
	Total individuals (log)	2.907	1.014	0.004

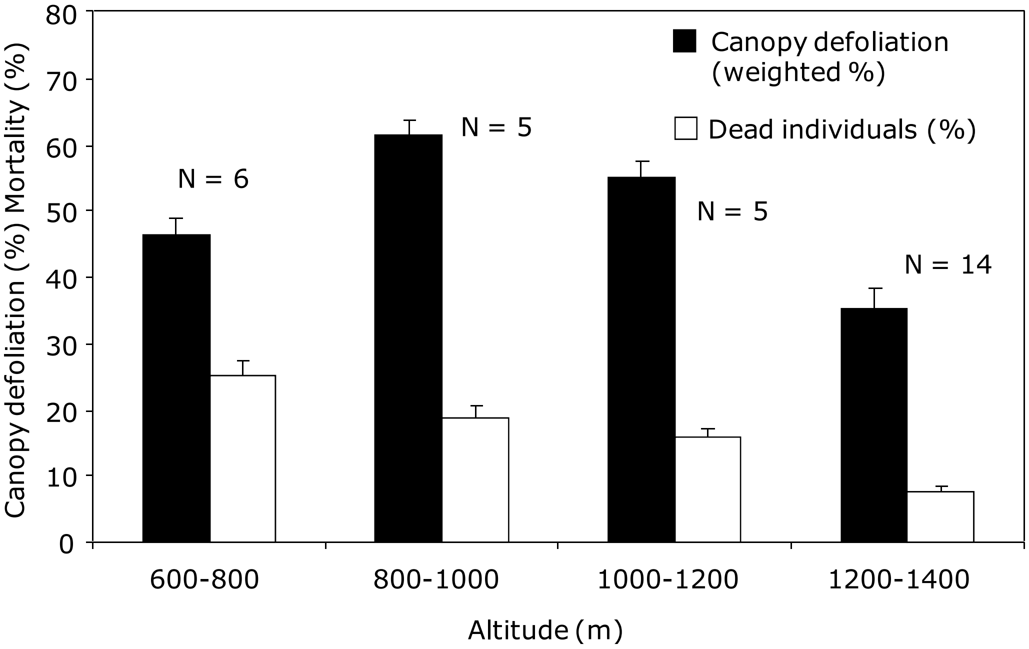

Figure 2.1. Percentage of canopy defoliation of living individuals and standing mortality (plot level) along the altitudinal gradient. Error bars show standard errors. The number of plots (N) at each altitude level is shown on top of the bars.

At the individual level, the stepwise model selection used to fit both defoliation and mortality models did not remove any explanatory variable. All the variables tested were significantly associated with defoliation and mortality (Table 2.4). Canopy defoliation and mortality were higher in smaller trees (lower DBH; see Figure 2.2) and with high intra-specific competition (larger values of Hegyi competition index; see Figure 2.2). Defoliation was also associated to mistletoe occurrence, with defoliation progressively increasing on more heavily infested trees (Table 2.4). High intensity of mistletoe infection tended to occur on larger trees (One-Way ANOVA, F = 51.927, P < 0.001; see also Figure 2.2), but within a given mistletoe occurrence class small trees were more defoliated than large ones (Figure 2.2).

Table 2.4. General and generalized linear mixed models for defoliation and mortality, respectively, at the individual level. A stepwise model selection was used in both cases starting from the same initial set of explanatory variables. Only the final models are shown. N=1002; $AIC_{defoliation\ model}$ = -151.414, N=856; $AIC_{mortality\ model}$ = 688.7.

	Model terms	β	SE	P
Defoliation individual-level model	DBH	-0.009	0.001	<0.001
	Hegyi competition index	0.002	0.001	0.006
	Mistletoe index-low occurrence	0.048	0.023	0.038
	Mistletoe index-medium occurrence	0.110	0.035	0.002
	Mistletoe index-high occurrence	0.130	0.039	0.001
Mortality individual -level model	DBH	-0.058	0.014	<0.001
	Hegyi competition index	0.036	0.009	<0.001

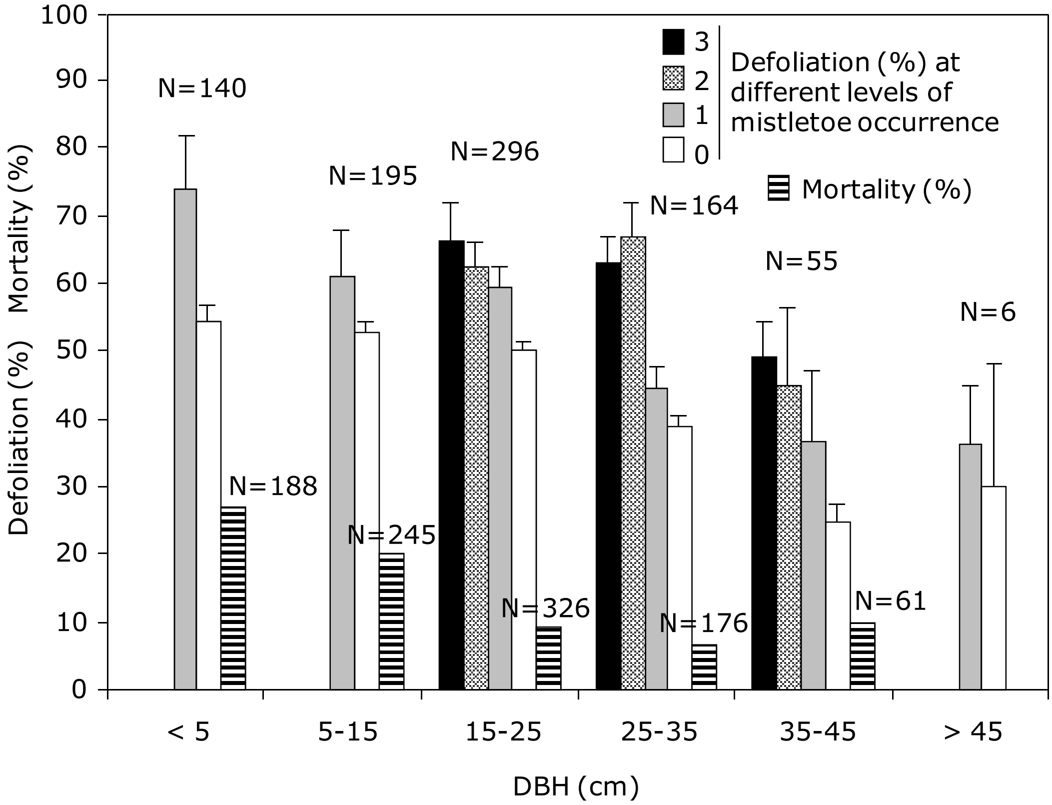

Figure 2.2. Distribution of defoliation and mortality across diameter classes in the studied *Pinus sylvestris* stands. Defoliation effects are split by mistletoe occurrence levels (0 = no, 1 = low, 2 = medium, 3 = heavy) for each diameter class. Errors bars show standard errors. The number of individuals (N) in each diameter class (including all mistletoe classes) for defoliation is shown on top of the defoliation bars. The number of individuals (N) used for mortality estimates is shown on the top of the mortality bars.

Community dynamics

At the plot level, a high recruitment of the shrub *Buxus sempervirens* was found in all plots (10250 seedlings/ha on average; Appendix 2.3). *Quercus humilis* and *Q. ilex* were the trees with higher recruitment in the study area, with 2483 and 1700 seedlings/ha on average, respectively. In contrast, we found an average abundance of 766 seedlings/ha for *P. sylvestris*, mostly concentrated in one single plot with a density of 13500 seedlings/ha (see also Figure 2.3a). Average *P. sylvestris* seedling abundance excluding this plot was 327 seedlings/ha.

Q. humilis and *Q. ilex* abundance were correlated to each other (Figure 2.3a; r = 0.755, P < 0.001). Overall seedling abundance of *Quercus* species tended to be higher at low altitudes (Figure 2.3a), in agreement with higher density of adults of these species towards the valley bottom (Figure 2.3b). Nevertheless, *Quercus* recruitment was also high at intermediate altitudes; despite that mature trees of *Quercus* species were almost absent (Figure 2.3b).

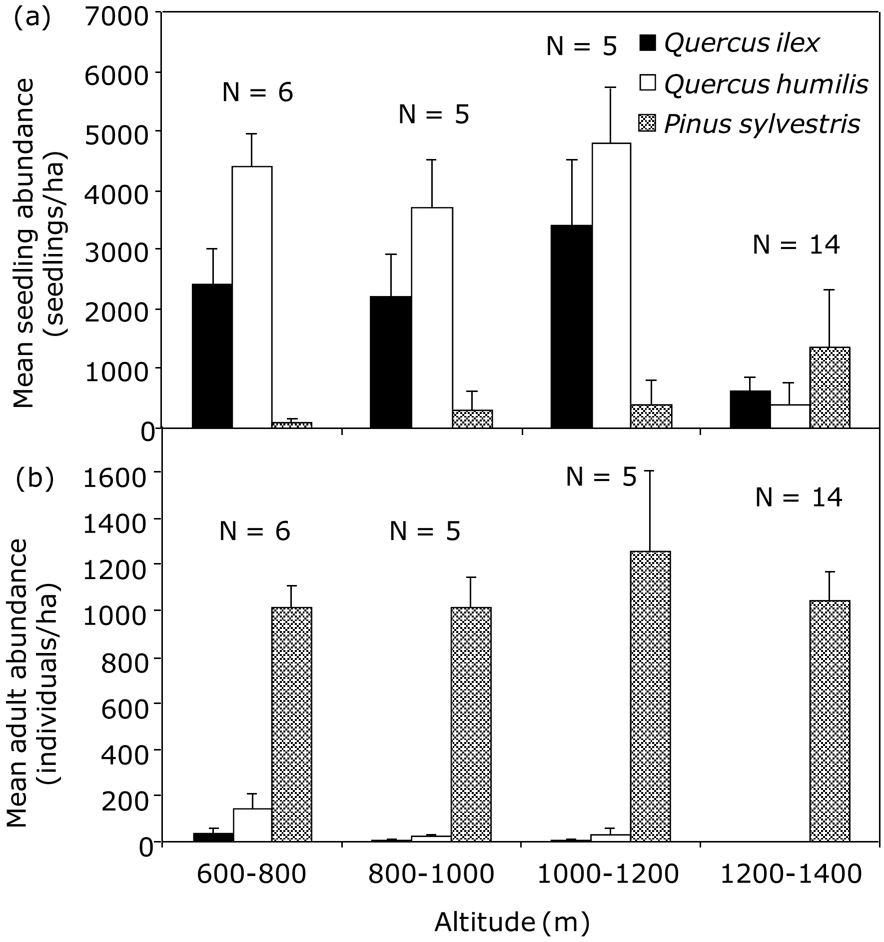

Figure 2.3. Seedling (a) and adult (b) abundance distribution of *Quercus ilex*, *Q. humilis,* and *Pinus sylvestris* along the altitudinal gradient (bars). Error bars show standard errors. The number of plots (N) at each altitude level is shown on top of the bars.

The stepwise model selection used to fit the recruitment model removed two explanatory variables: stand density and the Saxton *B* coefficient. Total canopy defoliation was the most significant variable in the recruitment model, with higher *Quercus* recruitment where defoliation was higher (Table 2.5). With regard to the variables linked to competition, mean *P. sylvestris* DBH was the most important in the model. Large values of DBH were related to lower seedling abundance of *Quercus* species. The aggregation index was marginally (and positively) related to seedling abundance, indicating that recruitment of *Quercus* species was higher where the adult trees of *P. sylvestris* were more uniformly distributed. The topographic wetness index and the depth of the soil were negatively associated with the seedling abundance of *Quercus* species. Finally, summer water availability was also negatively associated with the recruitment of *Quercus*, probably influenced by the absence of severe defoliation under relatively moist conditions (see Table 2.3).

Table 2.5. Generalized linear model for recruitment at the plot level. A stepwise model selection criterion was used. Only the final model is shown. N = 30 plots; $AIC_{recruitment}$ =165.82.

Model terms	β	SE	P
Summer water availability index	-4.640	2.325	0.046
Mean DBH	-0.097	0.036	0.007
Aggregation index	0.147	0.087	0.091
Topographic wetness index	-0.426	0.143	0.003
Soil depth	-0.021	0.009	0.023
Total canopy defoliation	0.049	0.009	<0.001

DISCUSSION

Determinants of defoliation and mortality

In addition to the expected association between forest decline (defoliation and mortality) and local level of drought stress, our results showed that such a relationship is complex and may involve a wide range of other contributing causes (Waring 1987; Franklin *et al.* 1987; Manion 1991). This is one of the few studies that have examined the many factors potentially associated with tree death, revealing the multifactor nature of drought-driven forest decline. The involved factors act differently on the dieback parameters: summer water availability and stand density were the most important driving factors of both mortality and defoliation, presumably acting during the 2004-2005 drought episode as inciting and long-term predisposing factors, respectively; whereas canopy defoliation was associated with additional predisposing factors such as soil properties and mistletoe infection. Thus, biotic as well as abiotic factors seemed to induce the first decline-symptoms of defoliation, but local drought conditions and stand structure were probably the factors that determined the mortality pattern.

Previous drought events surely initiated a lengthy chain of memory effects on trees (Bréda & Badeau 2008) that led to defoliation and mortality at the drier and lower altitudes within the studied valley during the severe 2004-2005 drought. During drought episodes, leaf-shedding could initially occur as an avoidance mechanism to maintain a favourable water balance by reducing transpiring needle area (Bréda *et al.* 2006). Nevertheless, leaf-shedding also signals the early stages of a sequence leading to tree death (Dobbertin 1999; Dobbertin & Brang 2001). In our case, stand-level mortality was positively correlated with defoliation recorded two years after the 2004-2005 drought event. This result suggests that the remaining trees did not take advantage of a release of competition for resources, probably because some sort of long-term drought effect occurred on the surviving trees (Bréda & Badeau 2008) and/or the inherently drier conditions impair the recovery of trees in those plots.

Water availability per unit of basal area is likely to be lower in plots with higher basal area (compare Callaway & Walker 1997; Briones *et al.* 1998). The relatively high tree density in the studied valley is likely the result of management abandonment during the last decades, as has been observed in other areas (Barbéro *et al.* 1998; Poyatos *et al.* 2003; Linares *et al.* 2009), and particularly of the reduction of logging practices in our *Pinus sylvestris* forests since the 1980s (Catalan Forest Service, Carles Fañanàs, pers. com.). The effect of increased aridity due to climate change occurs therefore on top of an ongoing process of "natural" self-thinning triggered by changes in forest management. Nevertheless, the absence of a significant interaction between the effects of drought stress and stand density for either defoliation or mortality suggests that competition and plot-level water availability have exerted additive effects on the studied *P. sylvestris* forest.

Soil properties, particularly soil texture and depth, were also associated with defoliation rates. Our results demonstrated that the least affected plots are distributed on deeper soils with more abundant clay. Clayey soils retain more water at a given water potential and cause a more gradual decrease in wetness as soil water content declines (Sperry *et al.* 1998). Other studies have associated fine-textured soils with milder effects of drought on plants (Hacke *et al.* 2000; Sperry & Hacke 2002; Hultine *et al.* 2005). The topographic wetness index did not improve the models explaining defoliation or mortality, possibly due to the relatively narrow range of topographic conditions occupied by *P. sylvestris* in the studied valley, which are constrained to steep slopes that remained unused by traditional agriculture (García-Ruiz *et al.* 1996; Poyatos *et al.* 2003).

Our results showed that defoliation was associated to mistletoe occurrence, with defoliation progressively increasing on more heavily infested trees. This is consistent with previous studies (Dobbertin & Rigling 2006), and with the fact that during a drought episode, when the tree reduces its transpiration rates by stomatal closure, the mistletoe continues to transpire increasing the water stress experienced by host trees (Fischer 1983; Press *et al.* 1988; Strong & Bannister 2002). Although we cannot rule out the possibility that defoliated trees are more prone to new mistletoe infections, the old age of most mistletoe individuals clearly suggests that infection occurred before 2004 in most cases, and likely acted as a predisposing factor during the 2004-2005 drought episode. High occurrence of mistletoe tended to appear on larger trees, probably because these trees contain large branches that provide perching and feeding sites for birds dispersing mistletoe seeds (Aukema & Martínez del Rio 2002). However, under the same mistletoe occurrence conditions, defoliation was observed preferentially on small trees, probably due to their lower rooting depth, and the corresponding disadvantage for belowground water competition (Pugnaire *et al*. 2000). Other studies have also documented major effects of drought on small trees (Martínez-Vilalta & Piñol 2002; Lloret *et al.* 2004; Smith *et al.* 2005; Bravo-Oviedo *et al.* 2005; Chen *et al.* 2008).

Community dynamics

Mortality of overstory trees has the capacity to alter the structure of the forest, including the regeneration environment. Light intensity and soil moisture conditions are key regulators of regeneration success (Pigott & Pigott 1993; Sack & Grubb 2002; Castro *et al.* 2004). In closed-canopy forests, gaps created when trees die promote high light intensity and a more xeric environment in the already drier stands (Suarez & Kitzberger 2008). In this study, the recruitment of *P. sylvestris* was very low in almost all plots, including those with high defoliation. This low recruitment contrasts with the high regeneration of pines in general and *P. sylvestris* in particular observed under open canopies (Keeley & Zedler 1998; Castro *et al.* 2004). Despite the shade-intolerant nature of *P. sylvestris* (Ceballos & Ruiz de la Torre 1971), seedlings require moderate shade to ensure certain soil moisture and air humidity (Schultz & Gatherum 1971; Karlsson & Nordell 1987; Broadmeadow & Jackson 2000; Castro *et al.* 2004).

Recent drought episodes may also have impaired directly the reproductive success of *P. sylvestris* in the study area. Some studies have reported lower cone production under drought (compare Zlotin & Parmenter 2008, for *Pinus edulis*; Mutke *et al.* 2005, for *Pinus pinea*) and under other stressful conditions that also entail defoliation (Hódar *et al.* 2003). Mistletoe infection has been also related with lower cone production and seed quantity and quality in pines (Schaffer *et al.* 1983; Singh & Carew 1989; Mathiasen *et al.* 2008). Loss of seed quality is likely to eventually limit the performance of *P. sylvestris* seedlings (Reich *et al.* 1994; Castro 1999).

Quercus humilis and *Q. ilex* were the trees with higher recruitment in the studied area. Interestingly, we found that the density of *Quercus* seedlings was higher in patches with increasing canopy defoliation, in agreement with the fact that seedlings of *Quercus* species are known to have a competitive advantage over *P. sylvestris* under drought stress conditions (Marañón *et al.* 2004). Unfortunately, we could not demonstrate that the 2004-2005 drought increased the emergence of new *Quercus* seedlings, because we do not know their age distribution in the study plots, and some seedlings may have been there before the drought event. Regardless of when the seedlings emerged, their current density was apparently increased by canopy defoliation, initiating the process of an eventual replacement of *P. sylvestris*. In the midterm, this trend could result in a vegetation shift in the study area, from pine-dominated to broadleaves-dominated forests.

Few studies have addressed the changes in recruitment patterns related to drought-induced mortality of the overstory vegetation. Van Mantgem & Stephenson (2007) analyzed mid-term changes in demographic rates of species growing in forests from southern USA. They found increased mortality rates in *Pinus* and *Abies* but no change in recruitment. We observed a differential pattern of seedling establishment favouring species that are not dominant under the current conditions. In general, the abundance of *Quercus* adult trees was very low in the studied slopes, and although they were mainly concentrated at low altitudes, the recruitment of *Quercus* species was also high at intermediate altitudes. Therefore, our results suggest that an altitudinal upwards migration of hardwood forests may take place in the studied area, as reported in other mountains of the region (Peñuelas & Boada 2003); noticeably, in our case this migration would be mediated by the drought-induced dieback of the dominant canopy species.

CONCLUSIONS

In conclusion, our study documents an episode of multifactor forest decline at the level of a single valley. At that scale, defoliation and mortality were mostly associated to the local water availability at each plot and to stand structure. The high mortality rates found in this study, together with the differential recruitment favouring species that are not dominant under the current conditions, have the capacity to transform the stand structure, composition and dynamics of the studied forests in the mid term. This trend observed at local scale supports biogeographical approaches revealing that many rear-edge populations of *P. sylvestris* sheltered in the mountain environments of the Iberian Peninsula could be at risk under future climate scenarios. At the same time, the relevance of stand structure in the observed pattern has important implications for forest management, as thinning treatments and sustainable harvest activities could potentially be used to mitigate the effects of climate change on high-dense stands (Millar *et al.* 2007).

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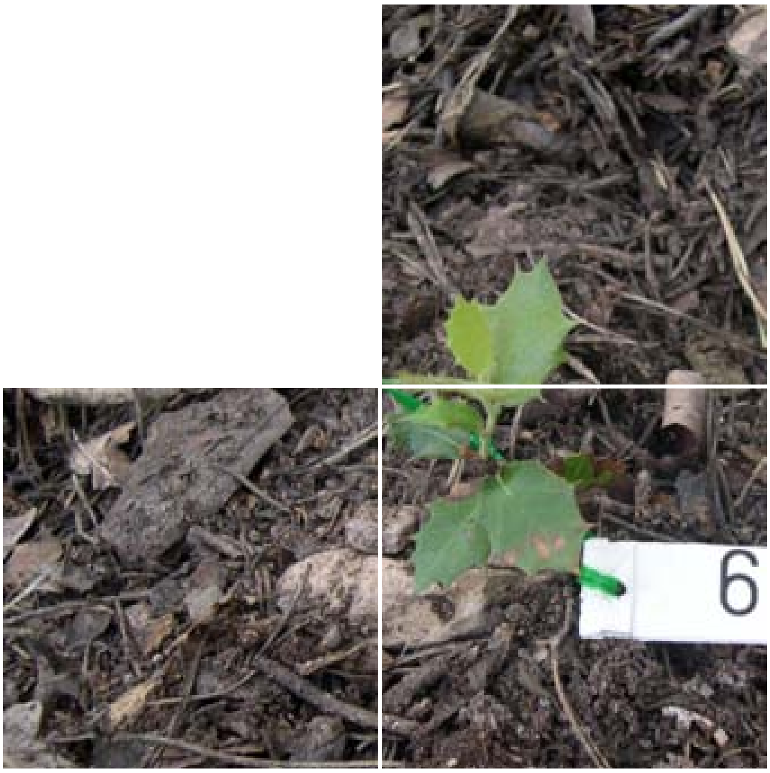

Capítulo 3

Seedling emergence and growth of *Quercus* spp. following severe drought effects on a *Pinus sylvestris* canopy

A modified version of this chapter has been submitted to *Journal of Vegetation Science*;
Galiano L, Martínez-Vilalta J, Eugenio M, de la Cerda IG and Lloret F (submitted)

RESUMEN

Emergencia y crecimiento de plántulas de *Quercus* spp. tras los efectos de una sequía severa en copas de *Pinus sylvestris*

Recientemente ha aumentado el interés por los efectos de las sequías extremas en relación a la mortalidad de la vegetación arbórea. Sin embargo, pocos estudios han analizado el impacto de las anomalías climáticas sobre las tendencias demográficas de las especies dominantes en el dosel forestal a más largo plazo, analizando los cambios en el reclutamiento. En este capítulo nos centramos en los patrones de reclutamiento de un bosque de *Pinus sylvestris* L. en los Pirineos centrales (NE España) que sufrió los efectos de una sequía severa en los años 2004-2005. Utilizamos un enfoque retrospectivo mediante el uso de la dendrocronología para analizar si un episodio de mortalidad inducida por sequía en la especie dominante (*P. sylvestris*) puede conducir a cambios en la vegetación. Nuestros resultados muestran que las pérdidas de dosel inducidas por sequía no se ven compensadas por la regeneración de *P. sylvestris*, mientras que otras especies arbóreas (*Quercus humilis* Mill. y *Q. ilex* L.) mostraron un elevado establecimiento en toda el área de estudio. Sin embargo, la relación entre la mortalidad de *P. sylvestris* y la regeneración de *Quercus* a nivel de parcela fue compleja. La abundancia de plántulas de *Quercus* fue mayor bajo doseles defoliados de pino, aunque esto no se debió a la emergencia de nuevas plántulas de *Quercus* después del episodio de sequía del 2004-2005, la cual fue incluso menor en lugares situados bajo dosel defoliado. El crecimiento de las plántulas de *Quercus* aumentó bajo doseles defoliados de pino, ilustrando que la dinámica forestal en condiciones de cambio climático podría acelararse debido a mecanismos distintos a la emergencia de plántulas de especies no dominantes. Junto con los cambios recientes en el clima, la aparente expansión de quercíneas hacia altitudes superiores en la ladera es probablemente el resultado del cese de las actividades silvícolas en los años 80. Puesto que las proyecciones climáticas apuntan a un incremento en la intensidad y la frecuencia de las sequías extremas, es esencial establecer parcelas de monitoreo que esclarezcan si los patrones que observamos en este estudio se mantendrán a largo plazo, pudiendo conducir finalmente a cambios en la vegetación.

ABSTRACT

Increased focus has been recently placed on the effects of extreme droughts on vegetation mortality. However, few studies have analyzed the impact of climate anomalies on longer term demographic trends of the dominant canopy species, accounting for recruitment. In this study we focus on recruitment patterns in a *Pinus sylvestris* L. forest in Central Pyrenees (NE Spain) that was affected by a severe drought in years 2004-2005. We use a retrospective, dendrochronological approach to analyze whether an episode of drought-induced mortality of the dominant species (*P. sylvestris*) may be acting as a driver of vegetation shift. Our results show that drought-induced canopy losses appear not to be compensated by regeneration of *P. sylvestris*, while other tree species (*Quercus humilis* Mill. and *Q. ilex* L.) showed high recruitment in the whole studied area. The association between plot-level mortality of *P. sylvestris* and *Quercus* regeneration was complex. The abundance of *Quercus* seedlings was higher under defoliated pine canopies, although it was not due to the emergence of new *Quercus* seedlings after the 2004-2005 drought, which was even lower under defoliated pine canopies. Growth of *Quercus* seedlings was enhanced under defoliated pine canopies, illustrating the existence of mechanisms different from seedling emergence of non-dominant species that could contribute to accelerate forest dynamics under climate change conditions. The apparent upward expansion of *Quercus* spp. in the studied slopes is likely the result of the cessation of species-selective forestry activities during the 1980s in combination with the recent changes in climate. Because of the predicted increases in the intensity and frequency of extreme droughts, monitoring studies are key to elucidate whether the initial patterns that we observed will be maintained in the long term, eventually leading to a vegetation shift.

INTRODUCTION

Drought-induced mortality episodes have been documented worldwide and have the capacity to cause rapid and large-scale vegetation shifts in woodlands and forest communities (Allen & Breshears 1998; Mueller *et al.* 2005; Allen *et al.* 2010). Accordingly, increasing efforts are being made to understand the many factors potentially involved in climate-related forest mortality (Suarez *et al.* 2004; Bigler *et al.* 2006; Galiano *et al.* 2010) as well as the underlying physiological mechanisms (McDowell *et al.* 2008; Sala *et al.* 2010; McDowell *et al.* 2011). By contrast, few studies have assessed the impacts of drought-induced mortality episodes on longer term demographic trends of the dominant canopy species, accounting for recruitment patterns (but see Van Mantgem & Stephenson 2007; Suarez & Kitzberger 2010).

Earlier studies show that differential mortality resulting from species-specific susceptibility to drought and subsequent reduction of propagule availability may not be a sufficient condition for long-term vegetation shifts (Suarez & Kitzberger 2008; Lloret *et al.* 2012). Of particular interest is the understanding of micro-scale environmental changes affecting tree regeneration performance after climate-related disturbances. Under closed-canopy forest, gaps created by drought-induced death of adult trees may provide new opportunities for regeneration. Seedling recruitment often compensates canopy losses of the dominant tree species as shown in studies of forest dynamics (Veblen 1992; Gray & Spies 1996; McCarthy 2001). However, drought disturbances create a particular type of canopy gap that may differ from the typical canopy opening resulting from tree falls (Clinton *et al.* 1993; Suarez & Kitzberger 2008) because: (a) the species-specific canopy tree mortality restricts the possible seeder's species that reach the regeneration bed; (b) the presence of dead standing trees that help maintaining relatively shaded microenvironments with relatively undisturbed soils tend to favor preexisting understory plants; (c) conditions tend to be more homogenous compared to tree-fall gaps due to the lack of coarse woody debris and the existence of diffuse gap boundaries; (d) overall, drought-induced gaps are particularly xeric due to the development of an understory that increases soil water uptake, and to the scarcity of coarse woody debris that would otherwise contribute to soil water retention. Specific studies are required to determine, in each particular case, whether the new conditions are more favorable for the establishment of new species and/or the development of understory species that may eventually lead to long-term vegetation shifts (see Clinton *et al.* 1993; Slik 2004; Suarez & Kitzberger 2008; Hiernaux *et al.* 2009).

Seedling recruitment is a critical process for the regeneration of plant populations because earlier development stages are frequently more vulnerable to environmental constraints than adult phases (Cook 1979; Fenner 1987; Silvertown & Lovett-Doust 1993). In woody plants, recruitment involves several sequential life-history stages connected by transitional processes (i.e. dispersal, emergence, survival, growth) (Grubb 1977; Harper 1977). Although many abiotic and biotic factors have been observed to disrupt the stage cascade by acting at some of these demographical transitions (seed arrival, light, soil physical and chemical properties, pathogens, herbivory, competition with herbs) (Schupp 1993; Davis *et al.* 1999; Nicotra *et al.* 1999; Herrera 2002), drought is considered a primary limitation to seedling establishment in many environments (Moles & Westoby 2004; Moss & Hermanutz 2009). Particularly, both experimental and natural drought conditions have been shown to negatively influence seed dispersal, seed banks, seedling emergence and performance (survival and growth) in Mediterranean ecosystems (Borchert *et al.* 1989; Dunne & Parker 1999; Castro *et al.* 2004; Lloret *et al.* 2005; del Cacho *et al.* 2011). Therefore, contrasted species-specific drought sensitivities at different life-history stages may lead to permanent shifts in community composition under future climate change scenarios (cf. Engelbrecht & Kursar 2003; Engelbrecht *et al.* 2005; Marañón *et al.* 2004; Lloret *et al.* 2012).

In this paper, we apply a retrospective approach based on seedling growth-rings to assess recruitment patterns as indicators of mid-term vegetation dynamics after an event of drought-induced forest die-off. In a previous study in Central Pyrennees (NE Spain), high densities of *Quercus ilex* L. and *Q. humilis* Mill. seedlings were observed beneath the canopy of *Pinus sylvestris* L. populations that experienced high defoliation and mortality rates as a result of a drought episode occurred in years 2004-2005 (Galiano *et al.* 2010). In contrast, the recruitment of *P. sylvestris* was much lower in highly defoliated stands, as has been also shown to be the case in general for *P. sylvestris* forests in the Iberian Peninsula (Vilà-Cabrera *et al.* 2011). As a result, *Quercus* spp. would have the potential to replace *P. sylvestris* in these declining forests. Here, we evaluate the age distribution and the radial growth of *Quercus* spp. seedlings under *P. sylvestris* canopies with different degree of defoliation and mortality to explore the relationship between drought-induced die-off and tree recruitment. Two main questions are addressed: (a) Did the 2004-2005 drought episode induce an increase in the emergence of new *Quercus* spp. seedlings under affected *P. sylvestris* individuals? (b) Was the radial growth of *Quercus* spp. seedlings promoted under defoliated *P. sylvestris* canopies as a result of the 2004-2005 drought?

MATERIAL AND METHODS

Study site

The study was conducted in a *Pinus sylvestris* forest located in the Central Pyrenees (Soriguera, Pallars Sobirà, 42º 22´ 43´´N, 1º 6´ 29´´E, ca. 16 km^2), mainly on northern slopes and at altitudes from 600 to 1500 m a.s.l. The shrub layer is predominantly occupied by *Buxus sempervirens* L., *Amelanchier ovalis* Medik. and *Lonicera xylosteum* L. Other tree species, *Quercus humilis*, *Quercus ilex* and *Betula pendula* Roth., occasionally appear in the understory, mostly at lower (*Quercus* spp.) and higher altitudes (*B. pendula*). A small *Pinus nigra* plantation interrupts the *P. sylvestris* forest at 1100 m approx. Soils are calcareous, fairly rocky (44 % weight of stones >1cm diameter) and belong predominantly to the clayey-loam texture class. The climate of the region is characterized by an annual mean temperature of 9.6 ºC and an annual mean rainfall around 637 mm (climate data obtained from the Spanish meteorological station network for the period 1951-2010, AEMET, Agencia Estatal de Meteorología), corresponding to the temperate oceanic submediterranean bioclimatic region (Worldwide Bioclimatic Classification System, 1996-2000).

Some stands in the studied *P. sylvestris* forest had traditionally been under important agro-pastoral pressure until the early twentieth century, and both natural and artificial afforestation have taken place in the area afterwards (Carles Fañanàs, Catalan Forest Service, pers. comm.), although the largest *P. sylvestris* trees in the stand are at least 150 years old. This population is within the natural distribution area of the species, but exhibits scarce natural regeneration (see Galiano *et al.* 2010). The studied *P. sylvestris* forest shows a stand density of 1071 individuals/ha and a basal area of 35.90 m^2/ha, and has not been managed since the 1980s. In 2004-2005, the Iberian Peninsula experienced a severe drought episode (EEA 2008), which was associated with high rates of defoliation and mortality in this *P. sylvestris* population (Galiano *et al.* 2010). Climate data show that more recent severe drought events have not occurred after the 2004-2005 episode (Appendix 2.2).

Field sampling

In June 2010, 14 and 13 square plots (3 $*$ 3 m each) were established under *P. sylvestris* canopies with < 50% and ≥ 50% of canopy defoliation, respectively. Plots with ≥ 50% of canopy defoliation included at least 25% standing dead trees in terms of basal area. On the other hand, dead trees in plots with < 50% of canopy defoliation corresponded to less than 5% of total basal area (Appendix 3.1), mostly suppressed small individuals. Canopy defoliation per plot was estimated by averaging the percentage of crown defoliation for all *P. sylvestris* trees (alive and dead; >50 cm height and >1.5 cm diameter at breast height, DBH) in a 5-m radius around the center of plots, weighting by the basal area of each tree. Plot selection used the following criteria in order to minimize unwanted environmental variation: no signs of recent disturbance, management or herbivory; distance between plots greater than 25 m; altitude from 900 to 1000 m a.s.l.; slopes from 10º to 40º and north-facing aspect. The altitude range for sampling was selected according to the upper altitudinal limit of *Quercus* spp. seedlings distribution that was previously found at the same site (Galiano *et al.* 2010).

All *Quercus ilex* and *Q. humilis* seedlings (<50 cm height and <1.5 cm diameter at the base of the stem) were inventoried and labeled in each of the plots. Total basal area was also computed for all living individuals (including all tree species; >50 cm height and >1.5 DBH) to assess the competition that adult trees may exert on seedlings. Age structure and growth patterns of the seedling population were estimated by harvesting the nearest four *Quercus* spp. seedlings to each plot side – in this way we avoided the destruction of former labeled seedlings which were maintained for future monitoring. Overall, 108 seedlings were harvested, and their root collars cut and kept into a tube containing a fixative solution (formalin-ethanol-acetic acid (5:90:5)). Since the studied *Quercus* spp. are well known resprouters, we excavated the root collar zone in each case to distinguish seedlings from sprouts emerging from preexisting root systems. For all seedlings, height and diameter at the base of the stem were measured in the field in order to estimate seedling volume (diameter^2 $*$ height).

We used the topographic wetness index developed by Beven & Kirkby (1979), which accounts for the contributing area that drains into a given point and for the slope of the terrain, to characterize within-catchment differences in soil water availability across plots. The topographic index was computed from a digital elevation model (DEM) with a resolution of 30 m, together with topographic slope recorded in the field at each plot. The topographic wetness index ranged from 7.22 to 11.38, with higher values representing wetter conditions. Understory light environment was quantified as canopy openness by using hemispherical photographs taken at a height of 1.35 m from the center of each plot. Photographs were taken with a digital camera (Nikon Coolpix 4300) using a leveled, north-oriented fisheye lens (Nikon Fisheye Converter FC-E8 0.21x) and were analyzed with the Gap Light Analyzer software (GLA version 2.0). Finally, the micro-scale environment of plots was characterized by the average percentage of superficial stones, herbs, moss and leaf litter estimated in 9 square subsections per plot.

Estimation of seedling age and growth

At the laboratory, histological micro-sections 10 to 15 µm-thick were obtained out of every root collar (N=108) by means of a sledge microtome (Sledge Microtome GSL1, Schenkung Dapples, Switzerland), placed on a slide and stained with Alcian Blue (1% solution in 0.7 N HCl) and Safranin (1% water soluble). That procedure results in unlignified and lignified cells appearing blue and red, respectively. Sections were afterwards dehydrated using a series of ethanol solutions at increasing concentrations, washed with xylol, and permanently embedded with Eukitt® glue (O. Kindler GmbH, Freiburg, Germany). Digital images of the best cross-section per seedling were captured using a Nikon D-90 digital camera mounted on a Nikon Eclipse 50i optical microscope x400 magnification. Establishment dates were estimated at an annual resolution by counting backwards from the outermost to the innermost ring. By convention, the year of establishment corresponds to the calendar year when the growing season started. Working on whole cross-sections minimized dating errors. However, ring series were too short to allow quantitative crossdating and 1- or 2-years dating errors are possible. Although evergreen species produce frequent double rings in Mediterranean areas (Cherubini *et al.* 2003), it becomes less probable in our studied submediterranean area due to the relatively low minimum temperatures achieved during the fall. A total of 14 seedlings were discarded for further analyses due to difficulties in identifying ring boundaries. Given the uncertainty in assigning precise dating and in order to increase the number of samples within each class, estimated establishment dates were grouped into six four-year classes: 2009-2006 (there was no seedling corresponding to year 2010), 2005-2002, 2001-1998, 1997-1994, 1993-1990, and before 1990. The percentage of these collected seedlings in each class was used to estimate the corresponding number of seedlings per class for all seedlings sampled in the plots (see Figure 3.1).

Additionally, two representative radii were selected per micro-section to measure ring widths and detect possible impacts of increased available solar radiation –as a result of drought-induced crown defoliation and mortality– on seedling growth. Measurements were performed to the nearest 0.001 mm using the WINDENDRO computer software (WINDENDRO 2004c, Régent Instruments Inc., Quebec, Canada). Basal Area Increment (BAI) was used to characterize seedling radial growth, and was calculated from ring growth according to: $\mathrm{BAI} = \pi \cdot \left(R_t^2 - R_{t-1}^2\right)$, where *R* is the radius of the tree and *t* the year of tree ring formation. As many seedlings were established before the 2004-2005 drought episode, post- (from 2006 to 2009) and pre-drought radial growth (from the establishment date to 2003) were calculated and compared for seedlings aged ≥ 10 years old (N=38). Growth for year 2010 was not considered because the growing season was not yet over by the end of the study.

Statistical analyses

At the plot level, a generalized linear model was used to assess the relationship between abundance of *Quercus* spp. seedlings and plot environmental characteristics. The pooled abundance of seedlings of both species in the plots was considered as a count response variable and errors were assumed to follow a Poisson distribution. All continuous explanatory variables included in the model were normally distributed or could be normalized using standard transformations: total basal area, topographic wetness index, superficial stones (ln transformed), herbs (ln transformed), moss and leaf litter (ln transformed). Canopy defoliation of *P. sylvestris* trees was included as a factor variable (< 50% and ≥ 50% of canopy defoliation). Canopy openness was not introduced into the model because it was highly correlated to canopy defoliation ($r = 0.728$, $P < 0.001$).

At the individual level, a general linear mixed model was used to explore the relationship between seedling age and volume accounting for individual plant and canopy defoliation characteristics. The response variable (volume) was normalized using a standard ln transformation. Regarding explanatory variables, age was normalized after standard ln transformation; species (*Quercus ilex* or *Q. humilis*) and defoliation of *P. sylvestris* (< 50% or ≥ 50% of canopy defoliation) were included as factors. With the aim of specifically assessing whether the relationship between volume and age varied according to the canopy defoliation class, we examined the interaction between age and *P. sylvestris* canopy defoliation. Plot effects were modeled as a random factor to account for the spatial autocorrelation among individuals within plots.

A general linear mixed model was used to test for post-drought growth effects (from 2006 to 2009) on *Quercus* spp. seedlings aging ≥ 10 years old. The response variable (post-drought BAI) was normalized using a standard ln transformation. As for the explanatory variables, age and pre-drought BAI (from establishment date to 2003) were normalized after standard ln transformation, and species (*Q. ilex* or *Q. humilis*) and canopy defoliation of *P. sylvestris* (< 50% or ≥ 50%) were included as factors. Age was introduced into the model because radial growth trends are related to increasing canopy volume in young trees (Weiner & Thomas 2001). As before, we examined the interaction between pre-drought BAI and *P. sylvestris* canopy defoliation. Plot effects were modeled as a random factor to account for the spatial autocorrelation among individuals within plots. Parameters (β) of all fitted models were estimated using maximum likelihood methods, and model selection was performed using AIC, so that we started from the saturated model and the least significant term was removed stepwise until no further reduction in AIC was observed. The residuals of all models were normally distributed.

A chi-square test was used to test for differences in the age-class distribution of *Quercus* spp. seedlings growing beneath *P. sylvestris* canopies with < 50% and ≥ 50% of canopy defoliation. Additional analyses were conducted using Pearson and Spearman correlation coefficients as a measure of association between pairs of variables. All statistical analyses were carried out with R version 2.12.0 (R Development Core Team, 2010).

RESULTS

Quercus humilis and *Q. ilex* were the tree species with higher recruitment in the narrow range of sampled altitudes (900-1000 m a.s.l.), with 3870 and 2140 seedlings/ha on average, respectively. For *P. sylvestris* we found an average abundance of only 165 seedlings/ha. The abundance of *Quercus* spp. seedlings was associated to the canopy defoliation of the overstory of *P. sylvestris* trees, with higher densities of *Quercus* spp. seedlings on more defoliated plots (Table 3.1). In addition, the abundance of *Quercus* spp. seedlings was higher in those stands with higher total basal area. Most of these seedlings (64%) belonged to *Q. humilis* and only 36% corresponded to *Q. ilex* individuals. Consistently, 72% and 28% of seedlings harvested in the ancillary sampling outside the plots corresponded to *Q. humilis* and *Q. ilex*, respectively. Explanatory variables illustrating the micro-scale environment of plots (ln[superficial stones], ln[herbs], moss and ln[leaf litter]) were removed from the final model because they were not significant and worsened the model fit in terms of the Akaike's information criterion (AIC).

Table 3.1. Generalized linear model of the abundance of *Quercus* spp. seedlings at the plot level (N = 27). Note: < 50% of *Pinus sylvestris* canopy defoliation was considered the reference level.

Model terms	β	SE	z	P
Intercept	1.917	0.614	3.118	0.002
Total basal area	0.00023	0.00006	3.57	<0.001
Topographic wetness index	-0.107	0.061	-1.760	0.078
P. sylvestris canopy defoliation ≥ 50%	0.425	0.216	1.968	0.049

The collected *Quercus* spp. seedlings had ages from 2 up to 34 years old, that is, establishment dates from 1977. Most seedlings (77%) established before or during the 2004-2005 drought episode. A chi-square test showed a marginal statistical difference between the age distributions of *Quercus* spp. seedlings growing under *P. sylvestris* canopies with < 50% and ≥ 50% of canopy defoliation, due to lower number of seedlings established in the post-drought period in highly defoliated sites (X^2 = 10.336, P = 0.066; Figure 3.1). Accordingly, a chi-square test considering *Quercus* spp. seedlings established before (all seedlings established before 2005) and after (from 2006 to 2009) the drought event clearly showed a higher emergence of new seedlings in less defoliated canopies after the drought episode (X^2 = 8.546, P = 0.003).

Our first general linear mixed model showed that, at a given age, the volume of *Quercus* spp. seedlings was higher under more defoliated (≥ 50%) compared to less defoliated (< 50%) *P. sylvestris* canopies (Figure 3.2 and Table 3.2). Accordingly, *Quercus* spp. seedlings showed a higher average radial growth in terms of BAI under *P. sylvestris* canopies showing ≥ 50% defoliation, becoming much higher after the 2004-2005 drought episode (Figure 3.3). The second general linear mixed model showed that, at a given pre-drought BAI, post-drought BAI was on average 0.23 mm^2 higher under highly defoliated compared to less defoliated *P. sylvestris* canopies (Figure 3.4 and Table 3.3). The interaction and species terms were removed from both final models because they were not significant and worsened the model fit in terms of AIC (see interaction details in Material and Methods section: Statistical analyses).

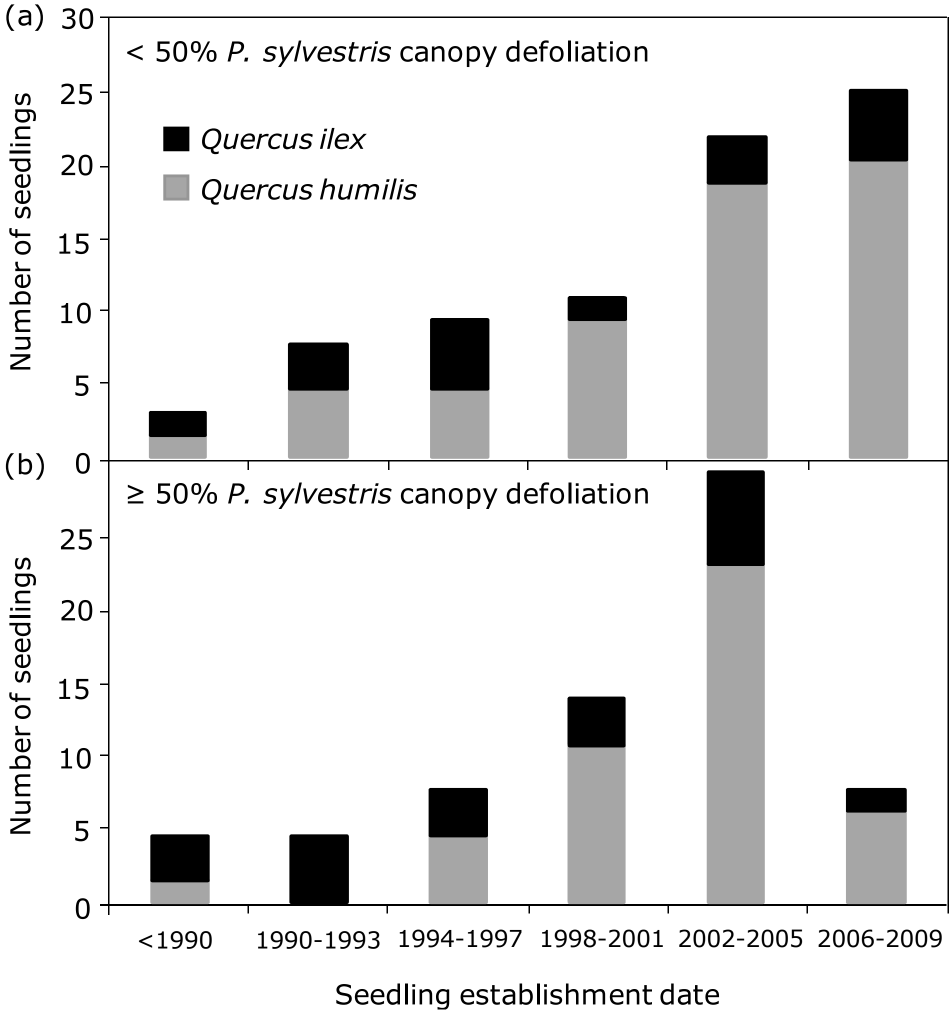

Figure 3.1. Frequency distribution of establishment dates of *Quercus ilex* and *Quercus humilis* seedlings growing under *Pinus sylvestris* canopies with (a) < 50% (N = 78) and (b) ≥ 50% (N = 68) of canopy defoliation. Establishment dates are binned in four-year periods. The sampled surface is 243 m^2. Marginal significant differences were detected between canopy defoliation classes (Kolmogorov-Smirnov two-sample test, P = 0.066).

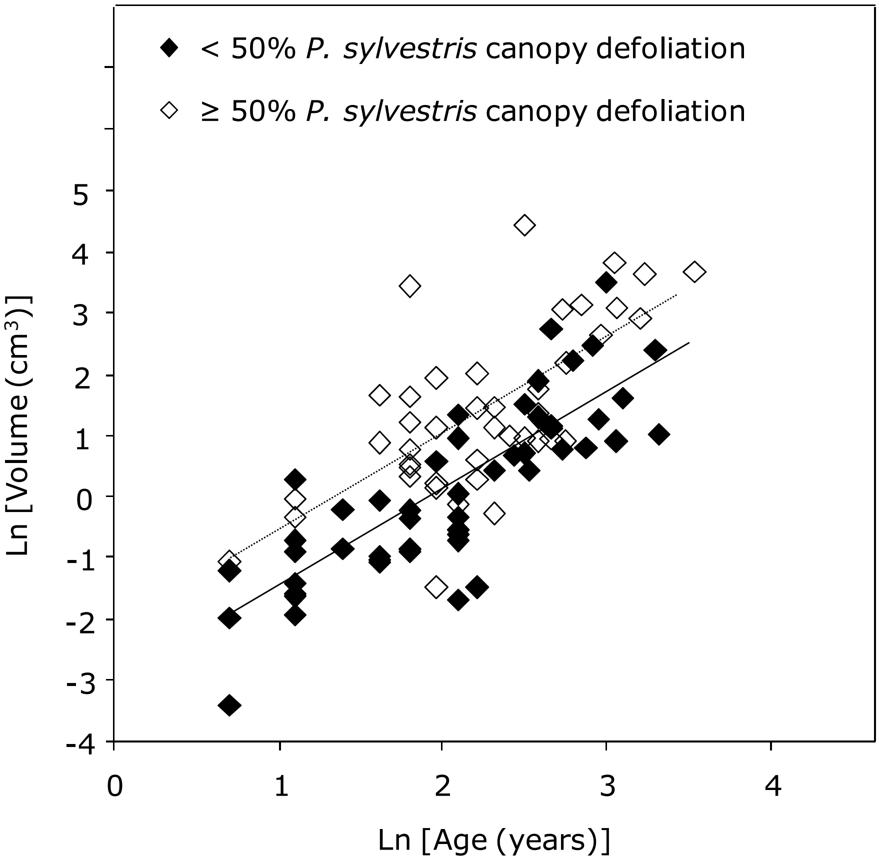

Figure 3.2. Relationship between ln(volume) and ln(age) of *Quercus* spp. seedlings growing under *Pinus sylvestris* canopies with < 50% (solid rhombus; r = 0.80 N = 50) and ≥ 50% (open rhombus; r = 0.61 N = 44) of canopy defoliation.

Table 3.2. General linear mixed model of the volume (Ln) of *Quercus* spp. seedlings at the individual level (N = 94). Note: < 50% was considered the reference level of the *Pinus sylvestris* canopy defoliation.

Model terms	β	SE	z	P
Intercept	-1.816	0.426	-4.264	<0.001
Ln [Age (years)]	0.851	0.190	4.471	<0.001
P. sylvestris canopy defoliation ≥ 50%	0.986	0.256	3.851	<0.001

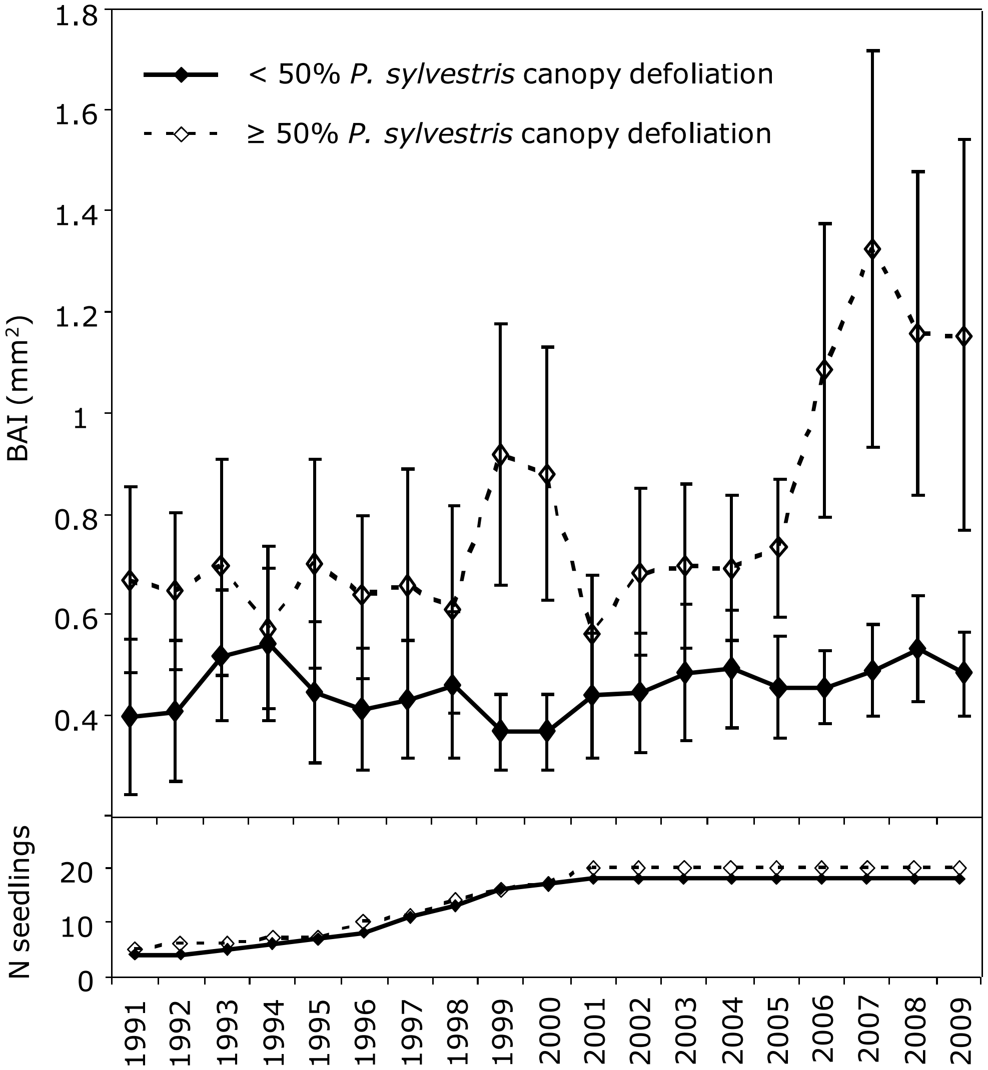

Figure 3.3. Temporal (1991-2009) growth trends (in terms of basal area increment, BAI) of *Quercus* spp. seedlings growing under *Pinus sylvestris* canopies with < 50% (solid line) and ≥ 50% (dotted line) of canopy defoliation. Error bars show standard errors.

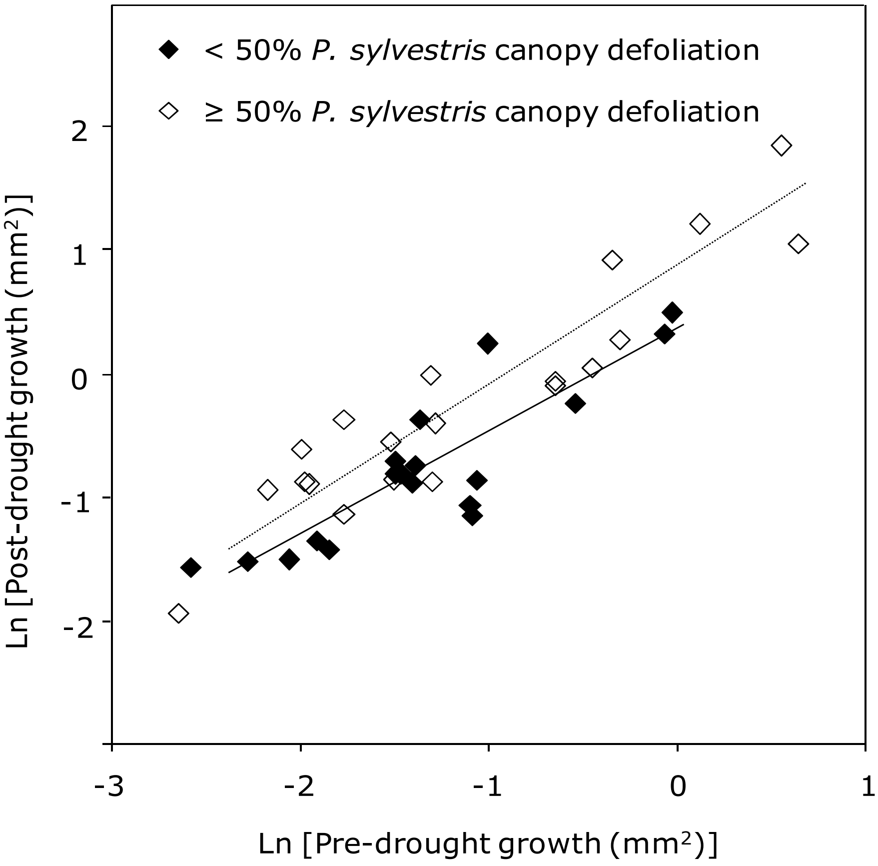

Figure 3.4. Relationship between the natural logarithms of post- (from 2006 to 2009) and pre-drought (from establishment date to 2003) radial growth (in terms of basal area increment, BAI) of *Quercus* spp. seedlings growing under *Pinus sylvestris* canopies with < 50% (solid rhombus; r = 0.84 N = 18) and ≥ 50% (open rhombus; r = 0.90 N = 20) of canopy defoliation.

Table 3.3. General linear mixed model of the post-drought growth (Ln BAI) of *Quercus* spp. seedlings at the individual level (N = 38). Note: < 50% was considered the reference level of the *Pinus sylvestris* canopy defoliation.

Model terms	β	SE	z	P
Intercept	-1.239	0.677	-1.83	0.083
Ln [Age (years)]	0.472	0.223	2.115	0.050
Ln [Pre-drought growth (mm²)]	0.731	0.082	8.931	<0.001
P. sylvestris canopy defoliation ≥ 50%	0.423	0.117	3.628	0.002

DISCUSSION

Droughts can cause widespread tree mortality in forests (Allen & Breshears 1998; Allen *et al.* 2010). However, the capacity of these events to induce vegetation shifts is eventually determined by the balance between the drought-induced mortality and the recruitment of the different species of the community (Lloret *et al.* 2012). In our case, canopy losses of the dominant species (*P. sylvestris*) were not compensated by its own regeneration (see also Galiano *et al.* 2010), indicating that the canopy openings resulting from drought-induced dieback were not enough to promote *P. sylvestris* regeneration (cf. Castro *et al.* 2004). This result is consistent with previous studies reporting drought-induced reductions in cone production (cf. Zlotin & Parmenter 2008, for *Pinus edulis*; Mutke *et al.* 2005, for *Pinus pinea*) and loss of seed quality (cf. García *et al.* 2000, for *Juniperus communis*).

In contrast, the recruitment of below-canopy tree species that could potentially become dominant (particularly *Q. humilis* and *Q. ilex*) was much higher than that of *P. sylvestris*. However, the spatial patterns of *Quercus* spp. regeneration resulting from the 2004-2005 drought were complex, with opposite patterns for the growth of preexisting seedlings and the establishment of new recruits. The canopy gaps created by the drought episode likely reduced light limitation, resulting in the radial growth release of the preexisting seedling bank, as was immediately detected the following years after the drought episode. The shade-tolerant nature of *Quercus* species allows seedlings to persist in the understory with very low growth rates until canopy opening creates new opportunities to develop (Espelta 2004). In our case, however, growth rates appear to be particularly low, since many individuals older than 20 years barely reached 0.5 m in height (cf. Espelta *et al*. 1995, also for *Quercus ilex*). Many studies have documented understory extension growth associated to forest canopy opening (Van Der Meer *et al.* 1999; Wilder *et al.* 1999), but studies on regeneration processes following extreme drought effects on the overstory are still scarce (Suarez & Kitzberger 2008). Higher growth rates confer competitive advantages to seedlings, including increased survival and reproductive outcome, which could facilitate the expansion of the species by eventually establishing new propagules (cf. Walters & Reich 2000).

In spite that the opening of canopy gaps benefit the growth of seedlings established before the drought, our results indicate that recent recruitment of *Quercus* spp. seedlings was higher under less defoliated canopies of *P. sylvestris*. These results suggest that the more xeric gap conditions originated by the drought episode may overwhelm the capacity of *Quercus* seedlings to face water stress during the early stages of establishment (Espelta 1996; Rey Benayas 1998; Retana *et al.* 1999). Several studies have shown contrasted sensitivities to drought of different life-history stages for *Quercus ilex*: higher seedling survival after summer drought has been observed for *Q. ilex* compared for instance to *P. sylvestris* (Marañón *et al.* 2004), but negative effects of increased light levels and associated decrease in soil moisture content have been shown for germination

(Bran *et al.* 1990; Broncano *et al.* 1998). Seed dispersal may also have been disrupted in patches with canopy gaps due to changes in dispersers' behavior (cf. Moran *et al.* 2004; Cramer *et al.* 2007; Zozaya *et al.* 2011).

Higher densities of *Quercus* spp. seedlings were observed in patches with higher *P. sylvestris* defoliation and mortality (cf. Galiano *et al.* 2010). Our results here indicate, in fact, that the higher density of *Quercus* spp. seedlings found in those sites was not due to the emergence of new seedlings after the 2004-2005 drought, but to higher initial seedling densities in the plots that ended up being more affected by the drought. This initial difference was likely related to the effect of basal area. Higher values of total plot basal area was positively related to higher abundance of seedlings, which suggest that the shade-tolerant nature of *Quercus* spp. could benefit from the shadier conditions provided by trees before the drought event. At the same time, stands with higher among trees competition suffered major defoliation effects during the drought episode (cf. Galiano *et al.* 2010), and the resulting canopy gaps did not provide appropriate conditions for new recruitment.

In the context of global change, land use changes have been documented as major drivers of compositional shifts in forest communities in general (Brown & Wu 2005; Gowda *et al.* 2012) and in the Mediterranean Basin in particular (Lookingbill & Zavala 2000; Romero-Calcerrada & Perry 2004; Urbieta *et al.* 2008), in addition to climatic disturbances. In the study area, the scarcity of adults and saplings of *Quercus* spp. with seedlings at most 30 years old, suggests that species-selective forestry activities suppressed *Quercus* spp. regeneration prior to the cessation of forestry management practices in the 1980s (Carles Fañanàs, Catalan Forest Service, pers. comm.). Since that date, expansion of shade-tolerant species, such as *Quercus ilex* and *Quercus humilis,* may have taken place at low and intermediate altitudes, whereas colder conditions likely prevented the establishment of these species at higher altitudes. This consideration, together with the higher altitudes reached by adults of *Quercus* spp. in southern aspects within the same valley, supports the idea that the warming observed over the last decades in the study area (Hereş *et al.* 2011) could be facilitating the upwards expansion of *Quercus* seedlings. Overall, our results suggest that different factors, directly and indirectly related to the effects of drought on the overstory, are contributing to the apparent altitudinal upwards expansion of *Quercus* species in the study area (cf McEwan *et al.* 2011).

In conclusion, our study highlights the complexity of forest responses to extreme droughts, and the importance of considering the interactions and synergies among different components of global change to explain drought-induced mortality episodes in forests and the subsequent regeneration processes. The altitudinal upwards expansion of *Quercus* species at the expense of *P. sylvestris* in the study area would be the result of a combination of recent climate warming, land use changes and response to extreme climatic episodes. Although historical human changes in land use and forestry activities would play an important role explaining the current distribution of *Quercus* spp. seedlings in the studied slopes, drought-induced mortality gaps provide new opportunities for regeneration. We demonstrate that while the emergence of new *Quercus* seedlings is prevented by drought-induced canopy openings, growth responses of a preexisting seedling bank could still contribute to accelerate forest dynamics under drier conditions. Long-term studies monitoring seedling establishment and growth, as well as delayed mortality of adults of all potentially dominant species are needed to confirm vegetation shifts in forests under enhanced climate-change type droughts.

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Capítulo 4

Carbon reserves and canopy defoliation determine the recovery of *Pinus sylvestris* four years after a drought episode

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RESUMEN

Las reservas de carbono y la defoliación del dosel determinan la recuperación de *Pinus sylvestris* cuatro años después del episodio de sequía

Los episodios de sequía severa pueden aumentar el estrés fisiológico de las especies leñosas de vida larga, ocasionalmente conduciendo a la mortalidad de los árboles del dosel. Sin embargo, se sabe poco sobre los factores que determinan la supervivencia de los árboles y la posterior recuperación tras la sequía. En este capítulo, utilizamos modelos de ecuaciones estructurales para analizar la recuperación de *Pinus sylvestris* L. cuatro años después de un episodio extremo de sequía que tuvo lugar durante los años 2004-2005 en el Pirineo occidental (NE España). Las variables que medimos incluyeron la cantidad de follaje verde, las reservas de carbono del tronco, la infección por muérdago, medidas del funcionamiento fisiológico de las hojas, y el crecimiento radial del tronco antes, durante y después del episodio de sequía. Los resultados mostraron que tanto el follaje verde como los niveles de reservas de carbono estaban relacionados con el impacto que tuvo la sequía sobre el crecimiento radial, y a su vez ambas variables estaban mutuamente correlacionadas. Sin embargo, nuestro modelo más probable indicaba que el agotamiento actual de las reservas de carbono era el resultado de la reducción de tejido fotosintético. En cualquier caso, esta relación constituye un potencial mecanismo de retroalimentación positiva que limita la recuperación de los árboles. Adicionalmente, la infección por muérdago reducía el contenido de nitrógeno en las hojas afectando negativamente el crecimiento. Finalmente, muestreos sucesivos del estado de los árboles en los años 2009 y 2010 mostraron una asociación directa entre el agotamiento de las reservas de carbono y la mortalidad inducida por sequía. Por lo tanto, se concluye que episodios de sequía severa pueden inducir trastornos fisiológicos a largo plazo asociados con la defoliación del dosel y el agotamiento de las reservas de carbono, dando lugar a recuperaciones prolongadas de los individuos supervivientes y, eventualmente, a la muerte de los árboles varios años después del episodio.

ABSTRACT

Severe drought may increase physiological stress on long-lived woody vegetation, occasionally leading to mortality of overstory trees. Little is known about the factors determining tree survival and subsequent recovery after drought. We used structural equation modelling to analyze the recovery of *Pinus sylvestris* L. trees four years after an extreme drought episode occurred in 2004-2005 in NE Spain. Measured variables included the amount of green foliage, carbon reserves in the stem, mistletoe infection, needle physiological performance, and stem radial growth before, during and after the drought event. The amount of green leaves and the levels of carbon reserves were related to the impact of drought on radial growth, and mutually correlated. However, our most likely path model indicated that current depletion of carbon reserves was a result of reduced photosynthetic tissue. This relationship potentially constitutes a feedback limiting tree recovery. In addition, mistletoe infection reduced leaf nitrogen content, negatively affecting growth. Finally, successive surveys in 2009-2010 showed a direct association between carbon reserves depletion and drought-induced mortality. Severe drought events may induce long-term physiological disorders associated with canopy defoliation and depletion of carbon reserves, leading to prolonged recovery of surviving individuals and, eventually, to delayed tree death.

INTRODUCTION

Episodes of drought-induced tree mortality are emerging as a global phenomenon and have already been reported from a variety of woodland and forest communities in many parts of the world (see review by Allen *et al.* 2010). Severe drought events may increase physiological stress on long-lived woody vegetation, occasionally leading to rapid defoliation and mortality of overstory trees (Bréda *et al.* 2006). In the Mediterranean basin, climate change effects interact with the effects of increasingly denser stands because of agricultural land abandonment (Poyatos *et al.* 2003), artificial afforestation (Martínez-García 1999) and a decline in logging practices (Linares *et al.* 2009; 2010). In addition, rear-edge populations are likely to be particularly sensitive to the effects of increased aridity (Hampe & Petit 2005). This pattern is well exemplified by Scots pine (*Pinus sylvestris* L.), a widely distributed tree species that has recently suffered episodes of drought-induced mortality in several of its southernmost populations (Martínez-Vilalta & Piñol 2002; Hódar *et al.* 2003; Galiano *et al.* 2010).

Two main physiological mechanisms explaining the eventual drought-induced mortality of trees have been recently formalized by McDowell *et al.* (2008): hydraulic failure and carbon starvation. According to these authors, the relevance of each mechanism depends on the hydraulic properties of the species and on the intensity and length of the drought conditions. Anisohydric plants maintain relatively high stomatal conductance as soil water potential decreases and are likely to die through hydraulic failure when the drought is particularly intense (Tyree & Sperry 1989; Davis *et al.* 2002; Brodribb & Cochard 2009). By contrast, isohydric plants prevent desiccation by stricter stomatal control, causing a decrease of photosynthetic carbon uptake and plant starvation as a result of continued metabolic demand for carbohydrates and depletion of reserves (Parker & Patton 1975; Martínez-Vilalta *et al.* 2002; Bréda *et al.* 2006). Isohydric plants are most likely to die during prolonged droughts of intermediate intensity. However, our current understanding of the prevalence of these mechanisms is still limited as they have proved difficult to demonstrate under natural conditions, and alternative mechanisms have been recently proposed (McDowell & Sevanto 2010; Sala *et al.* 2010).

Mature trees store large amounts of mobile carbon pools that are mostly composed of nonstructural carbohydrates (NSC) and neutral lipids (triacylglycerols). The size of this pool may be considered an indicator of a plant's "fuelling" status with respect to carbon, because it should reflect any shortage (depletion) or surplus (accumulation) depending on the carbon source-sink balance (photosynthesis vs metabolism, growth and export; Mooney 1972; Chapin *et al.* 1990; Körner 2003). Thus, stored carbon accumulated in any organ or tissue during periods of carbon surplus can be later mobilized to sinks where carbon demand temporarily exceeds available carbon (Chapin *et al.* 1990). It is well known that this pool may become smaller when sources are removed, for instance when trees are experimentally defoliated or pruned (Langström *et al.* 1990;

Vanderklein & Reich 1999; Kosola *et al.* 2001; Li *et al.* 2002) or may become larger when sinks are removed, for instance when trees are experimentally debudded (Chapin & Wardlaw 1988; Li *et al.* 2002). Drought normally impairs carbon assimilation through stomatal closure. However, drought also reduces carbon sinks, in particular, structural growth, so the relevant question is whether (or when) carbon sources are more constrained by drought than carbon sinks. In fact, the carbon reserves stored in woody plants have been shown to remain stable, increase or decrease in response to drought, depending on the species and their ontogenetic stage (Sala *et al.* 2010). In any case, although there is some indirect evidence consistent with the carbon starvation hypothesis (Adams *et al.* 2009; Breshears *et al.* 2009), direct evidence is completely lacking to date (Sala 2009).

Biotic agents such as pathogens and insects may intensify the negative effects of drought (Breshears *et al.* 2005; Desprez-Loustau *et al.* 2006; Dobbertin *et al.* 2007). Mistletoes are vascular hemiparasitic plants that may contribute to the water stress experienced by host plants during a drought, because they continue to transpire when trees reduce their transpiration rates by stomatal closure (Fischer 1983; Press *et al.* 1988; Strong & Bannister 2002). It is also well known that these hemiparasitic plants directly absorb other resources from their hosts (Glatzel 1983; Schulze & Ehleringer 1984; Ehleringer *et al.* 1986; Ehleringer & Marshall 1995). Photosynthetic rates of mistletoes are considered to be low (Stewart & Press 1990; Marshall & Ehleringer 1990) and several studies have demonstrated that a significant part of the carbon of mistletoes originates from the host plant (Marshall & Ehleringer 1990; Schulze *et al.* 1991; Marshall *et al.* 1994; Escher *et al.* 2004). Thus, mistletoe infection may induce numerous host physiological responses, such as lowered tree growth and altered resource allocation (Filip *et al.* 1993; Sala *et al.* 2001; Noetzli *et al.* 2003; Meinzer *et al.* 2004), and these responses may be enhanced under drought conditions (Dobbertin 2005; Stanton 2007).

Pinus sylvestris is a classical example of a species exhibiting isohydric stomatal control (Irvine *et al.* 1998). In a previous study of a *P. sylvestris* forest affected by a drought episode, occurred in 2004 and 2005, it was found that mortality and defoliation rates were associated with the local level of drought stress experienced at different altitudes. In addition, stand density, soil properties and mistletoe infection acted as long-term predisposing factors during the drought episode (Galiano *et al.* 2010). In the present study, we analyze the recovery of the previous *P. sylvestris* forest four years after the drought event. The main objectives were to: (1) establish whether the amount of carbon reserves stored in stems were related to the recovery of trees, and, more specifically, to the amount of green leaves; (2) test alternative hypotheses relating the impact of drought on trees, the amount of green leaves and the amount of carbon reserves stored in stems (see the bullet points at the end of the paragraph); (3) estimate how the previous relationships may be affected by mistletoe infection and by the physiological performance of current needles; and (4) investigate how the amount of carbon reserves may determine the capacity of trees to create new photosynthetic tissue and the survival of trees one year after the sampling. In relation to objective (2) we considered the following three hypotheses (cf., panels in Figure 4.1):

- Hypothesis 1: the current amount of green leaves, reduced by the impact of the past drought episode, causes photosynthetic carbon uptake to decline and, consequently, depletion of carbon reserves stored in stems.
- Hypothesis 2: there is a depletion of carbon reserves determined by their use during the past drought episode, proportional to the intensity of drought stress experienced by each tree. This depletion remains several years after the drought episode and reduces the current capacity of trees to create new photosynthetic tissue.
- Hypothesis 3: both the amount of green leaves and the levels of carbon reserves are reduced by the direct impact of the past drought episode. These two variables are mutually constrained and, consequently, they are positively correlated.

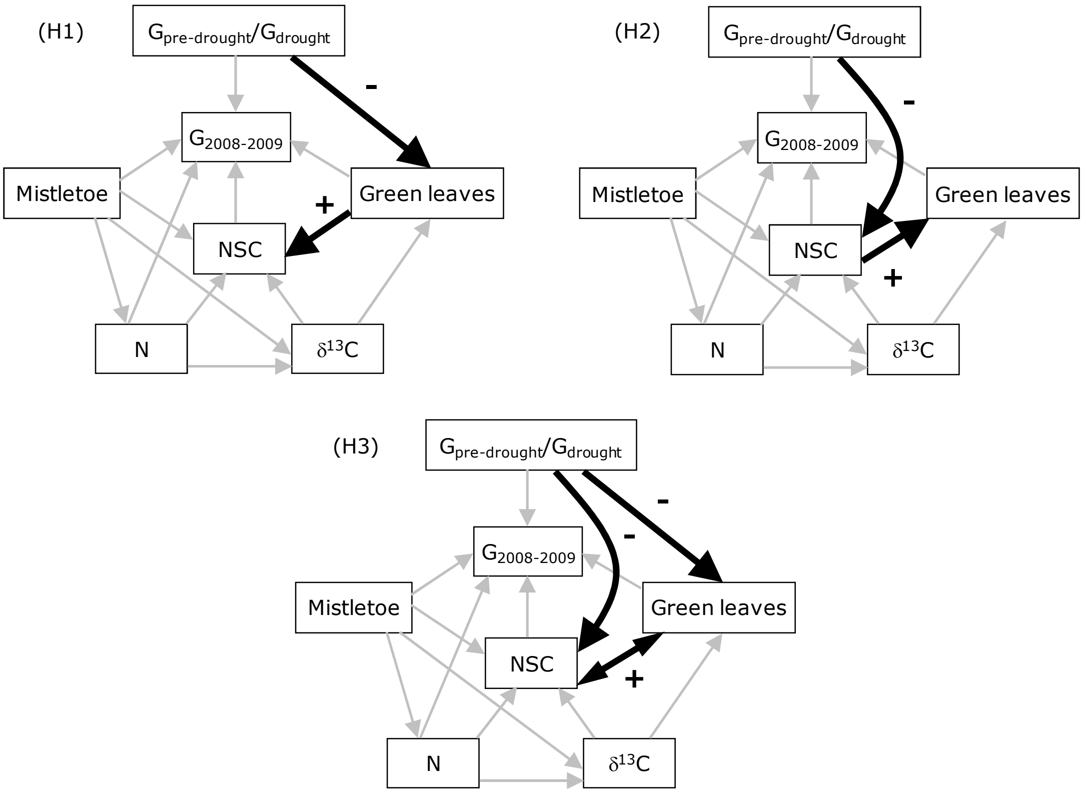

Figure 4.1. Three alternative hypotheses (H1, H2 and H3; see Introduction and Material and Methods: Statistical analyses) relating the impact of drought on trees ($G_{pre\text{-}drought}/G_{drought}$), the current growth rate ($G_{2008\text{-}2009}$), the amount of green leaves and the levels of carbon reserves stored in stems (NSC). These hypotheses are the basis of the structural equation models tested in this study (see Figure 4.4). Bold lines indicate the relationships determining each hypothesis. The effects of mistletoe infection and the physiological performance of current needles (leaf N content and leaf $\delta^{13}C$) on the previous relationships are also displayed.

MATERIALS AND METHODS

Study site

The study was carried out in a *Pinus sylvestris* forest located in the Central Pyrenees (Soriguera, Pallars Sobirà, 42º 22´ 43´´N, 1º 6´ 29´´E, ca. 16 km^2) with a stand density of 1071 individuals/ha and a basal area of 35.90 m^2/ha. *P. sylvestris* forests in the area are mainly on northern slopes, are distributed at altitudes from 600 to 1500 m a.s.l., and most of them have traditionally been under important agropastoral use up until the early twentieth century. Although both natural and artificial afforestation have taken place in the area during the twentieth century, the current *P. sylvestris* population is at least 150 years old, exhibits natural regeneration and is well within the natural distribution area of *P. sylvestris* (see Galiano *et al.* 2010). The *P. sylvestris* stands studied have not been managed since the 1980s (Carles Fañanàs, Catalan Forest Service, pers. com.). The shrub layer is predominantly occupied by *Buxus sempervirens* L., *Amelanchier ovalis* Medik. and *Lonicera xylosteum* L. Other species of trees, *Quercus humilis* Mill., *Quercus ilex* L. and *Betula pendula* Roth, occasionally appear in the understory, mostly at lower (*Quercus* species) and higher altitudes (*B. pendula*). Some younger *Pinus nigra* Arnold. plantations interrupt the *P. sylvestris* forest. A well-established mistletoe (*Viscum album* L.) population, with individuals up to 30 years old, infects most *P. sylvestris* trees in the area. Soils are calcareous, fairly rocky (44 % weight of stones >1cm diameter) and belong predominantly to the clayey-loam texture class.

The climate of the region is characterized by an annual mean temperature of 9.6 ºC and an annual mean rainfall around 643 mm (climate data obtained from the Spanish meteorological station network for the period 1951-1999, AEMET, Agencia Estatal de Meteorología), corresponding to the temperate oceanic submediterranean bioclimatic region (Worldwide Bioclimatic Classification System 1996-2009). In years 2004 and 2005, the Iberian Peninsula experienced a severe drought episode preceded by several dry periods during the last decades (EEA 2008; Appendix 2.2). Damage on *P. sylvestris* became visually evident in summer 2005 (Carles Fañanàs, Catalan Forest Service, pers. com.). This observation was supported by dendroecological analyses that showed lower growth and higher mortality in 2005 and subsequent years, although minor mortality episodes were also detected prior to 2005 (Hereş *et al.* 2011). At the time of sampling, average standing tree mortality was 14.3%, corresponding to 9.13% of total basal area, whereas defoliation (weighted by the basal area of each tree) was 45.18% (see Galiano *et al.* 2010). Climate data showed the absence of new severe drought events after the drought episode occurred in 2004-2005 (Appendix 2.2).

Field sampling methods

In August 2009, 42 *P. sylvestris* individuals were sampled. To distinguish between the effect of green needle amount and mistletoe parasitism on nonstructural carbohydrates (NSC) reserves stored in stem sapwood, we selected trees with contrasted amounts of green needles combined with different mistletoe infection intensities. We refer to the amount of green leaves instead of defoliation because our measure includes drought-induced defoliation and any recovery occurred afterwards. Tree selection used the following criteria in order to minimize unwanted variation: (a) diameter at breast height (DBH) from 15 to 40 cm; (b) no signs of recent disturbance or management; (c) distance between trees > 15 m; (d) altitude from 900 to 1000 m a.s.l.; (e) slopes from 10º to 40º and North aspect. For all individual trees, we visually estimated the percentage of green needles relative to the number in a healthy canopy of a similar sized tree in the study area. We minimized error by having the same person always observing the crown from the same position close to the stem and making the estimate separately for four different sections of the crown. Preliminary trials indicated that the average discrepancy between two trained, independent observers was <10%. Furthermore, DBH and a detailed characterization of all mistletoe plants based on age were recorded for all individual trees. The mistletoe characterization included six categories (1 = no bifurcations, 2 = 1-2 nodes, 3 = 3-5 nodes, 4 = 6-10 nodes, 5 = 11-20 nodes, 6 = > 21 nodes), accounting for the dichasial branching pattern of annual growth (Zuber 2004). A mistletoe index was calculated by adding the category of all mistletoe plants on each sampled tree. In addition, in July 2010, state (alive vs dead) and visual estimation of the percentage of green needles (%) was recorded again for all individual trees. We assessed the difference between the amount of green leaves in years 2010 and 2009. Larger positive differences imply greater recovery in the one year-long time lapse.

Using a hand increment borer (5 mm diameter, Suunto, Finland), two stem cores were sampled to the pith from each tree at 1.35 m above the ground from the two sides perpendicular to the slope. One core was used to measure NSC reserves stored in stem sapwood (see “Nonstructural carbohydrates” section), and the other core was used to quantify growth (see “Growth measurements” section). We also collected 20-30 exposed and apparently healthy current-year needles from two mid-canopy branches to analyze the carbon isotope composition and the nitrogen content (see “Foliar carbon isotope and nitrogen content” section).

Growth measurements

Cores for quantifying growth were placed in wooden supports and taken to the laboratory for analysis. In the laboratory, all cores were air dried and sanded using progressively finer sandpaper until growth rings could be easily recognized. The last thirty ring widths, that is, years from 1980 to 2009, were measured to a precision of 0.01 mm using the WinDENDRO computer software

(WinDENDRO TM 2004c, Régent Instruments Inc.). The COFECHA cross-dating program (Holmes 1983) was used to help detect the occurrence of missing and false rings. Two cores did not crossdate well and were excluded from further analyses. Basal area increment (BAI) was used to characterize tree growth. The BAI was calculated from ring growth according to: $\mathrm{BAI} = \pi \cdot \left(R_t^2 - R_{t-1}^2\right)$, where *R* is the radius of the tree and *t* the year of tree ring formation. To characterize the intensity of drought stress experienced by trees during the drought episode, we computed the ratio between the mean annual growth from 1980 to 2003 and the mean annual growth from 2004 to 2007. Growth from 2004 to 2007 was considered to be potentially affected by drought, as drought effects have been shown to last for several years in trees, including *P. sylvestris* trees (Becker 1989; Bréda *et al.* 2006). Larger values of this ratio indicate higher impact of drought on trees. We also computed the mean annual growth for years 2008 and 2009 to characterize the current growth rate.

Nonstructural carbohydrates

The stem sapwood portion of one core per tree (visually determined) was separated for measuring the nonstructural carbohydrates (NSC) reserves. The segments were wrapped in plastic straws and stored in a cooler over ice until sample processing in the laboratory on the same day. All sapwood samples were microwaved for 90 s to stop enzymatic activity, oven-dried for 72 h at 65 ºC, and ground to fine powder. NSC were defined as free sugars (glucose and fructose), low molecular weight sugars (free sugars and sucrose) plus starch, and were analyzed following the procedures described by Hoch *et al.* (2002), with some minor modifications. Approximately 12-14 mg of sapwood powder was extracted with 1.6 ml distilled water at 100 ºC for 60 min. After centrifugation, an aliquot of the extract was used for the determination of low molecular weight sugars after enzymatic conversion of fructose and sucrose into glucose. Another aliquot was incubated with an amyloglucosidase from *Aspergillus niger* at 50 ºC overnight, to break down all NSC (starch included) to glucose. The concentration of free glucose was determined photometrically in a 96-well microplate reader (Sunrise$^{\mathrm{TM}}$ Basic Tecan, Switzerland) after enzymatic conversion of glucose to gluconat-6-phosphate. Starch was calculated as NSC minus low molecular weight sugars. All NSC values are expressed as percent dry matter.

Foliar carbon isotope and nitrogen content

Current year needles were oven-dried for 72 h at 65 ºC and ground to fine powder. Ground samples were analyzed for carbon stable isotope composition and nitrogen content at the Cornell Isotope Laboratory (COIL) at Cornell University, using a Thermo Delta V isotope ratio mass spectrometer (IRMS) interfaced to a NC2500 elemental analyzer. The carbon stable isotope composition was expressed in delta notation: $\delta^{13}\mathrm{C}$ (‰) = ($R_{sample}/R_{standard}$ – 1) x 1000, where R_{sample} is the $^{13}\mathrm{C}/^{12}\mathrm{C}$ ratio of the sample and $R_{standard}$ is the $^{13}\mathrm{C}/^{12}\mathrm{C}$ ratio of the international Vienna

Pee Dee Belemnite carbon standard. The accuracy of the δ^{13}C measurements was 0.05 ‰. Nitrogen concentrations are expressed as percent dry matter.

Statistical analyses

We used structural equation modeling (SEM) to analyze the complex relationships between the set of observed variables representing the impact of drought on trees, the current growth rate, the amount of green leaves and the levels of carbon reserves stored in stems as well as how the previous relationships may be affected by mistletoe infection and by the physiological performance of current needles (leaf N content and leaf δ^{13}C; see Fig. 4.1). The SEM was performed by using AMOS 18 (Arbuckle 2009). Some variables were transformed to achieve normality. Because some variables remained nonnormal after transformation, the generalized least squares (GLS) method was used to estimate the value of the unknown parameters of the model, as recommended in cases of lack of multivariate normality and small sample sizes (Iriondo *et al.* 2003). Model selection was performed using the Bentler's comparative fit index (CFI), the Akaike's information criterion (AIC), and the Bayesian information criterion (BIC). Some additional analyses were carried out with R version 2.11.1. (R Development Core Team 2010) using the Mann-Whitney-Wilcoxon or Student's t and Kruskal-Wallis tests to compare means between two or more than two groups, respectively.

The relationships displayed in the structural equation models reflect the main hypotheses of this study (cf. Introduction; Fig. 4.1). The ratio between the mean annual growth from 1980 to 2003 and the mean annual growth from 2004 to 2007 is an estimator of the intensity of drought stress experienced by trees during the drought episode. This ratio is expected to influence the current growth rate (mean growth in years 2008 and 2009). We expected that the amount of green leaves also explains the current growth rate. Mistletoe infection has been shown to be directly connected to current water use efficiency (leaf δ^{13}C), stem sapwood NSC concentrations, leaf N content and current growth rate (Schulze & Ehleringer 1984; Ehleringer *et al.* 1986; Ehleringer & Marshall 1995; Escher *et al.* 2004). Leaf N content was directly connected to leaf δ^{13}C and stem sapwood NSC concentrations on the basis that nitrogen concentration is strongly associated with Rubisco concentration in leaves and, consequently, with carboxylation efficiency (Field & Mooney 1986). As leaf δ^{13}C was used as an integrated measure of the ratio photosynthetic capacity to stomatal conductance (water-use efficiency, Farquhar *et al.* 1989), the direct relationship between leaf N content and NSC concentrations allowed us to distinguish the effects of leaf δ^{13}C on carbon reserves via photosynthetic efficiency or via stomatal conductance. DBH was not introduced in the models because preliminary analyses showed no relationship between DBH and stem NSC concentrations, possibly due to the relatively narrow range of DBH considered in this study.

RESULTS

Trees with less than 50% of green leaves four years after the drought episode (2009) contained 34% less carbon reserves stored in stems than trees with more than 50% of green leaves (Mann-Whitney-Wilcoxon $W = 118$, $P < 0.05$; Figure 4.2a). In 2010, 47% of trees had increased the amount of green leaves with respect to 2009, whereas 28% and 24% had maintained and decreased the amount of green leaves, respectively. Similarly, trees with less than 50% of green leaves in 2009 presented 70% lower growth rates in years 2008 and 2009 with respect to those trees with more than 50% of green leaves (Student's $t = -4.321$, $P < 0.001$; Figure 4.2b). Interestingly, amounts of carbon reserves analyzed in 2009 were positively associated to the recovery of green leaves one year later (2010), as shown by significant differences between trees that increased and those that decreased the amounts of leaves (Kruskal-Wallis $X^2 = 7.681$, $P < 0.01$; see Figure 4.3a). It is also remarkable that the four trees that died between 2009 and 2010 had much lower stem NSC concentrations in 2009 than those that survived (Mann-Whitney-Wilcoxon $W = 3$, $P < 0.001$; Figure 4.3b). Sapwood area of trees was not related to NSC concentrations ($r = 0.065$, $P = 0.685$) and, thus, differences in NSC concentrations between defoliated/undefoliated trees and between dead/living trees were not caused by differences in sapwood area.

All SEM models present the following significant relationships (see Figure 4.4 and Appendix 4.1): (1) the current growth rate (mean growth in years 2008 and 2009) was negatively influenced by the direct impact of the past drought and the reduction of photosynthetic tissue, and by the indirect impact of mistletoe infection through the reduction of leaf N content; (2) leaf N content and leaf $\delta^{13}C$ were positively related (see Appendix 4.2). Despite this positive relationship, the fact that the current water-use efficiency (estimated from leaf $\delta^{13}C$) was negatively related to NSC concentrations, and there was no relationship between leaf N content and NSC, suggests an important role of stomatal closure in determining stem carbon reserves.

In addition to the previous relationships, the SEM model that most adequately describes our data was model (a), consistent with hypothesis H1 ($X^2 = 7.381$, $P = 0.390$, $CFI = 0.985$, $AIC = 49.381$, $BIC = 85.872$; Figure 4.4a), explaining 45% of the total variability of NSC concentrations. According to this model, the current amount of green leaves was reduced by the impact of the past drought episode and determined the current amount of carbon reserves. Thus, the impact of the drought on trees has an indirect effect on NSC concentrations, mediated by the amount of green leaves (see Appendix 4.1a). However, the SEM model corresponding to the hypothesis H3 also provided an acceptable fit ($P > 0.05$, $CFI > 0.9$, $\Delta AIC \approx 1$ and $\Delta BIC \approx 3$ compared with model (a); see Figure 4.4c). Thus, we cannot discard the possibility that carbon reserves were also constrained to some extent by the direct impact of the past drought episode and that their relationship with the amount of green leaves may be reciprocal. Model (b) was clearly worse than any of the two other models (CFI < 0.9, ∆AIC ≈ ∆BIC ≈ 3 units; see Figure 4.4b).

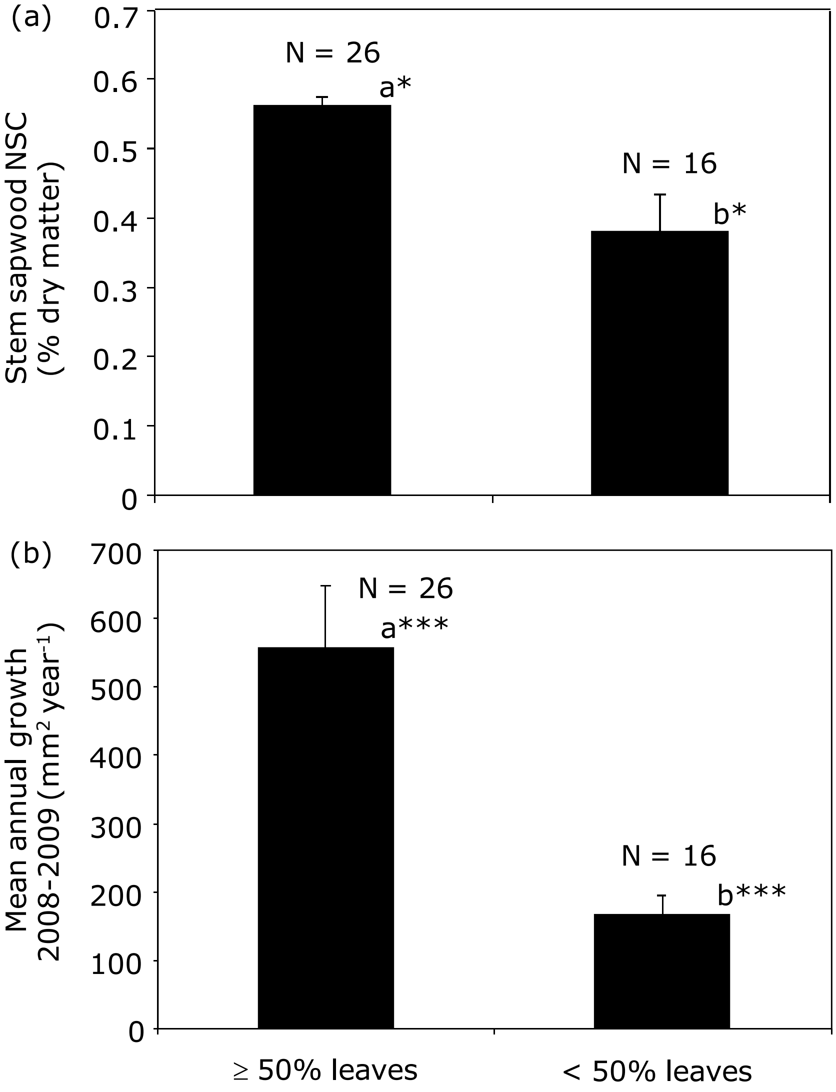

Figure 4.2. NSC concentrations stored in stems (a) and mean annual growth for years 2008 and 2009 (b) of two groups of trees with different amounts of green leaves (≥ 50% and < 50% of a healthy canopy) recorded in year 2009. Different letters indicate statistically significant differences (Mann-Whitney-Wilcoxon and Student's t tests, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$). Error bars show standard errors. The number of trees (N) for each group is shown on top of the bars.

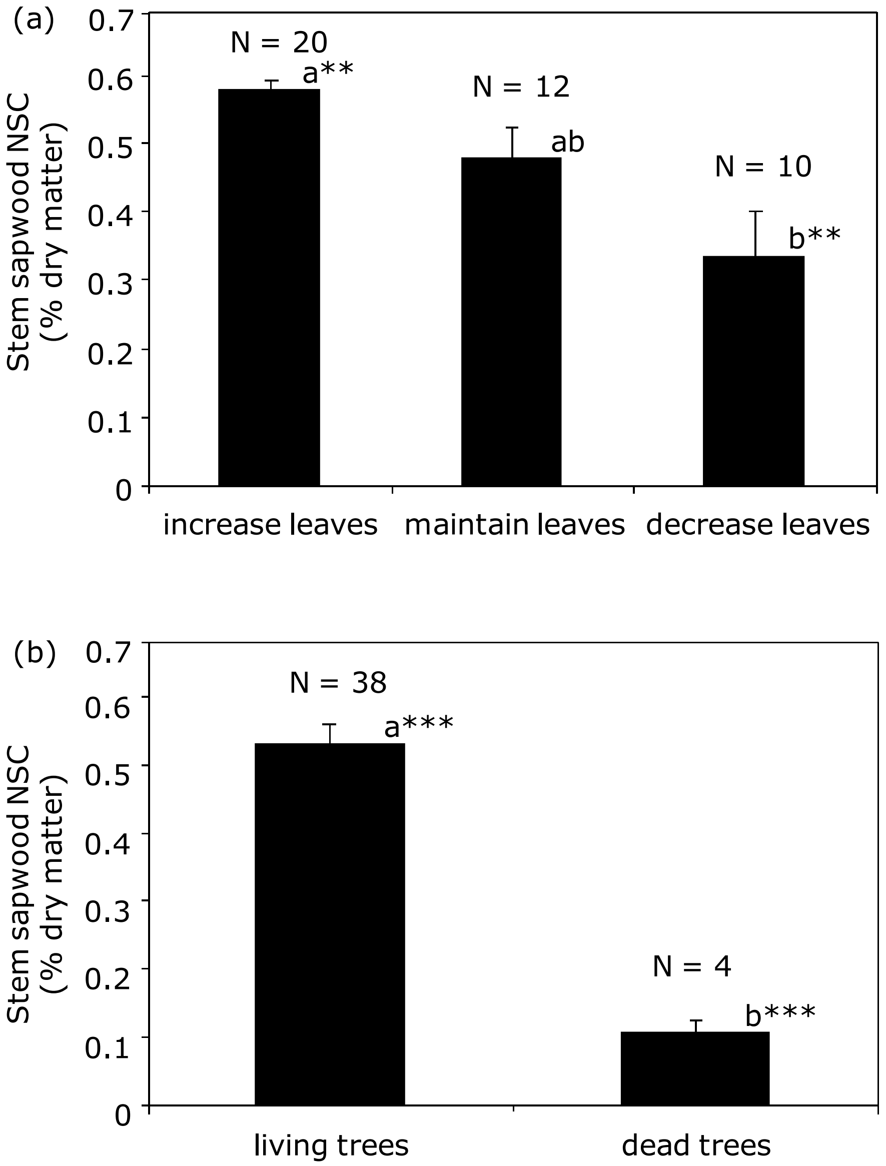

Figure 4.3. NSC concentrations stored in stems in 2009 (a) of three groups of trees depending on whether they increased, maintained or decreased the amount of green leaves between 2009 and 2010; and (b) of trees that died or survived between 2009 and 2010. Different letters indicate statistically significant differences (Kruskal-Wallis and Mann-Whitney-Wilcoxon tests, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$). Error bars show standard errors. The number of trees (N) for each group is shown on top of the bars.

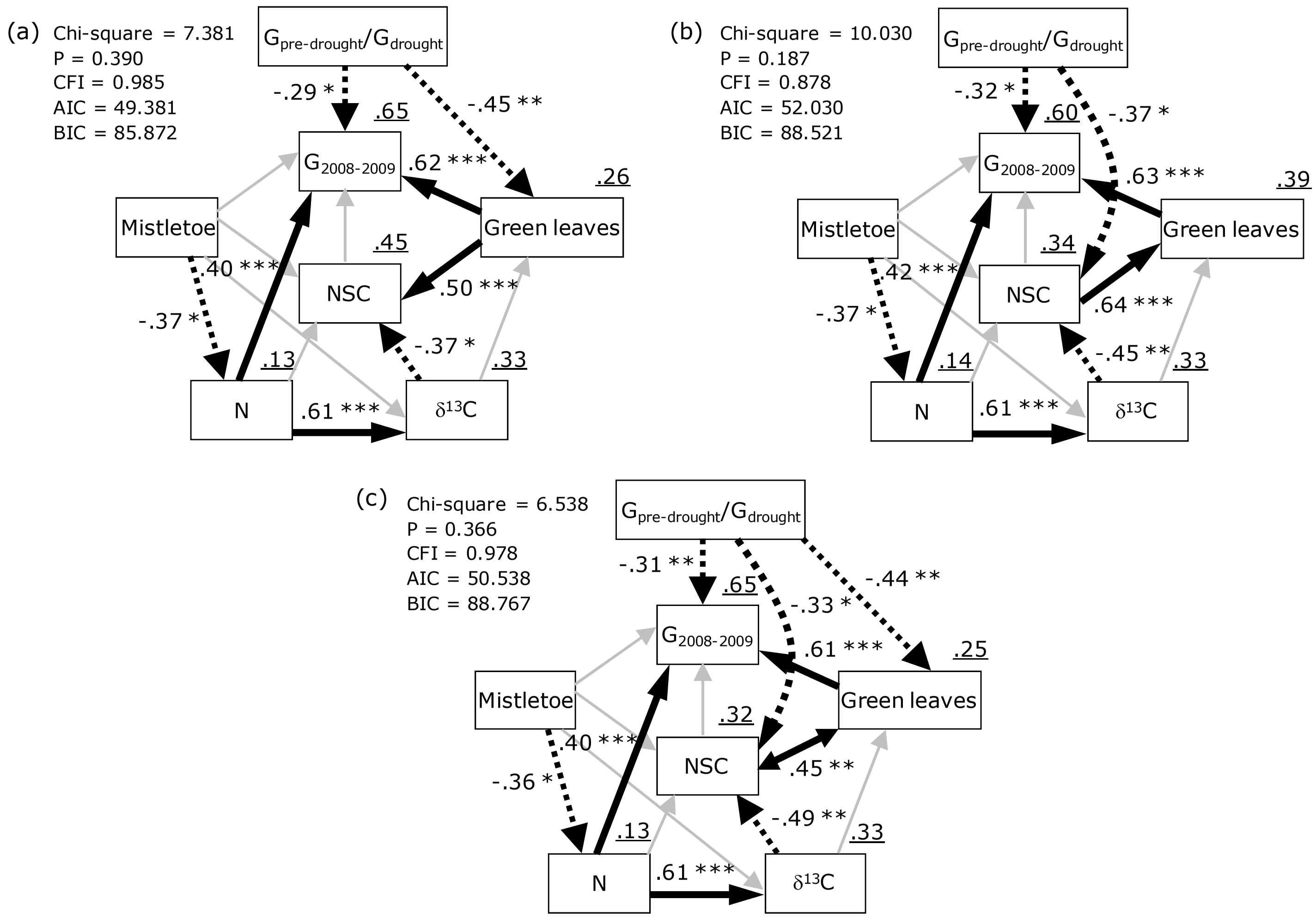

Figure 4.4. Results of the structural equation models representing three alternative hypotheses ((a) for hypothesis H1, (b) for hypothesis H2, and (c) for hypothesis H3), and showing the set of observed variables in boxes. Only the values of the path coefficients that were significant in the models are shown next to their corresponding arrow. Positive effects are indicated by solid lines, and negative effects by broken lines. The underlined values above response variable boxes indicate the explained variances (R^2). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

DISCUSSION

Pinus sylvestris trees in the studied area are still recovering from a drought episode occurred in 2004 and 2005. Carbon reserves stored in stems are related to the recovery of pine trees and, particularly, to the amount of green leaves. Trees that failed to recover more than 50% of green leaves contain very low levels of carbon reserves. Indeed, the entire *P. sylvestris* forest studied contained very low amounts of carbon reserves compared to other healthy *P. sylvestris* forests in southern Europe (Hoch *et al.* 2003).

Determinants of *Pinus sylvestris* drought recovery

Our SEM results showed that the current amount of green leaves was reduced by the impact of the past drought episode and support the notion that depletion of carbon reserves occurs primarily as a result of reduced photosynthetic carbon uptake, caused both by reductions in leaf area and by stomatal closure. Water scarcity during drought reduces stomatal conductance, leaf area, radial growth, and bud production (Tyree *et al.* 1993; Bréda *et al.* 2006; Pichler & Oberhuber 2007). The impairment of buds may limit the capacity of trees to create new photosynthetic tissue and twigs during the subsequent years following the drought episode (Power 1994; Stribley & Ashmore 2002). However, the reduction of leaf area is a well known mechanism of acclimation to water shortage in *P. sylvestris* (Martínez-Vilalta *et al.* 2009), and has been shown to induce growth reductions by limiting carbon assimilation in other pine species (e.g., Borghetti *et al.* 1998). It should be noted that our design does not allow separating the immediate impact of drought on leaf area (via needle shedding) from the delayed impact arising from, for example, the effects of drought on subsequent bud production.

Our results could not exclude the possibility that levels of carbon reserves were also constrained to some extent by the direct impact of the past drought episode. In agreement to the carbon source-sink balance theory (Mooney 1972; Chapin *et al.* 1990; Körner 2003), the depletion of carbon reserves in our case suggests that carbon assimilation (source activities), mainly impaired by leaf-shedding and stomatal closure, was more constrained than carbon sink activities as a result of the drought episode. The reciprocal relationship between the amount of green needle and carbon reserves may constitute a feed-back that limits the recovery of trees and explains the delayed effects of drought on tree growth and survival, which have been reported elsewhere (e.g., Manion 1991; Bréda *et al.* 2006; Bigler *et al.* 2007).

Despite the fact that several studies have reported that mistletoes extract carbohydrates from their hosts (Watson 2001; Escher *et al.* 2004), we did not observe any effect of mistletoe infection on the carbon reserves stored in stems. Instead, mistletoe absorption of nutrients noticeably reduced leaf nitrogen content, probably causing a reduction in the photosynthetic rate of pine needles (Meinzer *et al.* 2004). Mistletoe infection, thus, may reduce growth by limiting carbon assimilation rather than by reducing long-term carbon reserves or by changing the water balance of the tree. Other studies have also documented growth reductions in hosts caused by mistletoe infection (Dobbertin 2005; Rigling *et al.* 2010). Although our sampling design (cf. “Field sampling methods” section) did not allow us to test the well-known relationship between mistletoe infection and needle loss (Dobbertin & Rigling 2006; Rigling *et al.* 2010; Galiano *et al.* 2010), it is likely that mistletoe infection had also an indirect impact on tree growth, mediated by the amount of green leaves.

Implications for the mechanisms of drought-induced mortality

Repeated surveys in 2009 and 2010 allowed us to detect four trees that died in this one-year-long time lapse. These trees contained extremely low levels of carbon reserves in 2009. Consistent with this, surviving trees that exhibited less favorable canopy recovery from 2009 to 2010 also had lower levels of carbon reserves in 2009. These results show for the first time a direct association between carbon reserves depletion and drought-induced mortality in trees, and are consistent with the carbon starvation hypothesis (cf. McDowell *et al.* 2008). Previous evidence in favor of the carbon starvation hypothesis (Adams *et al.* 2009; Breshears *et al.* 2009) was indirect and consistent with alternative interpretations (Sala 2009; Leuzinger *et al.* 2009). Adams *et al.* (2009), for instance, found that higher temperatures in a period of protracted water stress increase respiration rates and accelerate mortality. Our study contributes to the current debate on the role of carbon reserves depletion on tree mortality (cf. McDowell & Sevanto 2010; Sala *et al.* 2010) by explicitly measuring carbon reserves stored in stems and showing that an isohydric tree such as *P. sylvestris* may actually deplete their carbon reserves and starve to death.

Although stomatal closure is normally described as the foremost mechanism that limits carbon uptake in the context of the carbon starvation hypothesis (e.g., Breshears *et al.* 2009), in our case the reduction in photosynthetic area owing to drought-induced leaf-shedding seems to be at least as important, as already suggested by McDowell *et al.* (2008). In any case, our observations on the mechanism of mortality remain somewhat preliminary, as our study was not designed to identify the mechanisms of drought-induced mortality in trees, and we cannot discard that other mechanisms, different from the carbon starvation hypothesis, might have been in operation. More research is needed to establish the causal relationships linking the dynamics of carbohydrate reserves and mortality, and to determine whether our results are generalizable to other species or situations.

In conclusion, our study illustrates how a severe drought episode can produce a progressive loss of forest resilience by depleting the ability of surviving plants to grow and survive future stressful events (Lloret *et al.* 2004), despite the release in competition that normally accompanies episodes of drought-induced mortality (Martínez-Vilalta & Piñol 2002; Bigler *et al.* 2006). Our results add to the evidence provided by earlier studies showing that long-term physiological disorders induced by a drought event occasionally lead to prolonged recovery phases of the surviving trees and/or eventual death (Bréda *et al.* 2006), and points to carbohydrate reserves as a key determinant of both tree survival and recovery.

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Capítulo 5

Determinants of prolonged drought effects on crown condition and their relationship with depletion of carbon reserves in a Mediterranean holm oak forest

A modified version of this chapter is in review in *Tree Physiology*;
Galiano L, Martínez-Vilalta J, Sabaté S and Lloret F (in review)

RESUMEN

Determinantes de los efectos prolongados de la sequía sobre la condición de las copas y su relación con el agotamiento de las reservas de carbono en un bosque mediterráneo de encina

Los episodios de sequía severa pueden aumentar el estrés fisiológico de las especies leñosas de vida larga, ocasionalmente conduciendo a una rápida defoliación y al progresivo aumento de mortalidad en los árboles del dosel. En los últimos años, se han documentado episodios de mortalidad inducida por sequía en una gran variedad de zonas forestales del mundo. Sin embargo, aun se sabe poco sobre los factores que determinan la supervivencia de los árboles y la posterior recuperación tras la sequía, especialmente en especies rebrotadoras. En este capítulo hemos estudiado los efectos prolongados de un episodio de sequía que tuvo lugar en el año 2000 sobre una población de encina (*Quercus ilex* L.) localizada en el NE España. Se utilizaron modelos lineales generalizados para analizar las correlaciones de variables ambientales con la condición de las copas del bosque siete años después del episodio. Adicionalmente, evaluamos la asociación entre la condición de las copas y las reservas de carbono y nutrientes almacenadas en el lignotuber siete años después del episodio de sequía. Nuestros resultados revelan la naturaleza multifactorial del declive del bosque observado en el que la profundidad del suelo y las características de los individuos, en particular el número de tallos, determinaron un patrón espacial complejo de respuestas a nivel de individuo. Este declive se asociaba con un agotamiento de las reservas de carbono en el lignotuber siete años después del episodio, con una reducción del 60% en los árboles que habían sido más dañados. Muestreos sucesivos del estado de los árboles en los años 2007 y 2011 mostraron una relación directa entre niveles bajos de las reservas de carbono y el deterioro adicional del estado de las copas en ausencia de nuevos episodios de sequía severa. Por lo tanto, se concluye que las cada vez más frecuentes sequías apuntadas por las proyecciones de cambio climático pueden conducir al progresivo agotamiento de las reservas de carbono y a la pérdida de resiliencia de las especies rebrotadoras mediterráneas.

ABSTRACT

Severe droughts may increase physiological stress on long-lived woody vegetation, occasionally leading to rapid defoliation and progressive increase in mortality of overstory trees. Over the last few years, episodes of drought-induced tree dieback have been documented in a variety of woodlands and forests around the world. However, the factors determining tree survival and subsequent recovery are still poorly understood, especially in resprouter species. We have studied the prolonged effects of a single drought episode, occurred in summer 2000, on a holm oak (*Quercus ilex* L.) forest located in NE Spain. Generalized linear models were used to study the environmental correlates of forest crown condition seven years after the drought event. Additionally, we evaluated the association between crown condition and the carbon and nutrient reserves stored in lignotubers seven years after the drought. Our study reveals the multifactor nature of a drought-driven forest dieback in which soil depth and the characteristics of individual trees, particularly their number of stems, determined a complex spatial pattern of tree-level responses. This dieback was associated with a depletion of the carbon reserves in lignotubers seven years after the episode, representing a reduction of up to 60% in highly drought-damaged trees. Interestingly, in the absence of new acute droughts, successive surveys in 2007-2011 showed a direct association between carbon reserves depletion and further deterioration of crown condition. More frequent droughts, as predicted by climate change projections, may lead to a progressive depletion of carbon reserves and to a loss of resilience in Mediterranean resprouter species.

INTRODUCTION

Severe drought events associated with global climate change may increase physiological stress on long-lived woody vegetation, occasionally leading to rapid defoliation and mortality of overstory trees (Bréda *et al.* 2006). Recent episodes of drought-induced tree dieback have been reported worldwide from a variety of woodland and forest communities (see review by Allen *et al.* 2010). Tree dieback is a complex process normally involving a wide range of potential causes (Waring 1987; Franklin *et al.* 1987) that some authors have framed in the context of the decline-disease theory (Manion 1991; see also Bigler *et al.* 2006 and Galiano *et al.* 2010). The importance of these widespread mortality events is reinforced because they have the capacity to transform the stand structure and dynamics, and the ecosystem functioning of regional landscapes on a sub-decadal timescale (McDowell *et al.* 2008).

In Mediterranean-type climates, drought has been recognized as the main factor limiting plant species growth and distribution (Mooney 1983; Terradas & Savé 1992). In the Mediterranean Basin, forest ecosystems may be particularly challenged if drought periods become even more frequent and intense (Borghetti *et al.* 1998; Martínez-Vilalta & Piñol 2002; Ogaya *et al.* 2003; Sarris *et al.* 2007), as predicted by climate change projections (Christensen *et al.* 2007; IPCC 2007). In recent decades, coupled to the negative consequences of increased drought, changes in human use of many Mediterranean forests have resulted in denser stands due to agricultural land abandonment (Poyatos *et al.* 2003; Romero-Calcerrada & Perry 2004), artificial afforestation (Martínez-García 1999; Navarro *et al.* 2010) and a decline in logging practices (Terradas 1999; Linares *et al.* 2009, 2010). Denser stands normally result in stronger plant competition for resources because of the corresponding reduction in soil water availability per tree, potentially exacerbating the vulnerability of forests to water stress (Bigler *et al.* 2006; Linares *et al.* 2009; Vilà-Cabrera *et al.* 2011).

Mediterranean-type ecosystems have been documented as being highly resilient to disturbances (Malanson & Trabaud 1987). The resprouting ability adopted by many Mediterranean trees and shrubs to recover after disturbance (Canadell & Zedler 1995) is a key element of the resilience of these communities (sensu Westman 1986). Many Mediterranean woody species have a large lignotuber – a woody swollen structure at the stem base (James 1984) –, that acts as a reservoir of dormant buds, carbohydrates and nutrients to ensure rapid regrowth after disturbances (Mullette & Bamber 1978; Canadell & Zedler 1995). However, severe disturbances may produce loss of resilience by depletion of reserves in surviving organs, particularly when new disturbances occur before the reserves are fully recovered (Jones & Laude 1960; Rundel *et al.* 1987; Malanson & Trabaud 1988). In fact, a progressive loss of resilience produced by recurrent disturbances in Mediterranean ecosystems has already been reported (Díaz-Delgado *et al.* 2002; Lloret *et al.* 2004). Although several studies have examined the dependence of resilience on the replenishment rates of carbon reserves after experimental disturbances (e.g. logging; Canadell & López-Soria 1998; López *et al.* 2009), less is known about its response to drought under natural conditions.

The evergreen holm oak (*Quercus ilex* L.) is a classical example of a Mediterranean species regenerating by resprouting from lignotubers after disturbance. Although the largest populations of this species occur in the western part of the Basin (Barbéro *et al.* 1992), it is present over a large area extending 6000 x 1500 km, from Portugal to Syria and from Morocco and Algeria to France (Terradas 1999). Holm oak is considered a typical Mediterranean drought-resistant tree species (Canadell & Rodà 1991; Savé *et al.* 1999; Infante *et al.* 2003). Nevertheless, holm oak trees has been observed to experience relatively low leaf water potentials (Limousin *et al.* 2009), high losses of xylem conductivity (Tognetti *et al.* 1998; Martínez-Vilalta *et al.* 2003) and negative carbon balances (Gracia *et al.* 2001) under extreme water stress conditions. In fact, drought-related holm oak dieback has already been reported in the Mediterranean Basin, showing that this species is more vulnerable to drought than other co-occurring species (Lloret & Siscart 1995; Peñuelas *et al.* 2000; Peñuelas *et al.* 2001; Martínez-Vilalta *et al.* 2002; Lorenz *et al.* 2005).

In this study, we analyze the prolonged effects of a single drought episode on a holm oak forest located in NE Spain seven years after the drought event. The main objectives were to: (1) examine the environmental correlates of forest crown condition emphasizing the role of forest intrinsic factors such as topographic conditions, stand structure and soil depth; (2) evaluate whether the amount of carbon and nutrient reserves stored in lignotubers as well as the physiological performance of current leaves were related to the crown condition of trees; and (3) explore whether the amount of carbon and nutrients stored in lignotubers may determine the changes of crown condition four years after the initial sampling. In relation to the two latter objectives we hypothesized that, seven years after the drought episode, stored carbon reserves would not be completely recovered in highly damaged trees and so we predict that those trees would be less able to cope with new drought events.

MATERIALS AND METHODS

Study site

The study was carried out in a holm oak (*Quercus ilex* L.) forest located in NE Spain (SW Garrotxa region, 42º8’43’’ N, 2º27’41’’ E, ca. 92 km^2) mainly on east, south and western slopes and distributed at altitudes from 500 to 900 m a.s.l. Holm oak is the dominant tree species at the study site, but a number of other typically Mediterranean species occasionally appear in the understory (*Pistacia lentiscus* L., *Juniperus oxycedrus* L., *Arbutus unedo* L., *Viburnum tinus* L., *Phillyrea latifolia* L., *Erica arborea* L.). The shrub layer is predominantly occupied by *Buxus sempervirens* L., *Rosmarinus officinalis* L. and *Cistus* spp. L. This forest was intensively coppiced for charcoal and firewood production until the late 1970s (Agelet & Montserrat 2002). According to López *et al.* (2009) we expect that the lapse since that date has been long enough to allow the recovery of carbon reserves by the year of the studied drought episode (2000). This type of forests shows extremely high stem densities due to natural regeneration from stump resprouting (Ibañez *et al.* 1999), while natural seedling recruitment is typically very low (Espelta *et al.* 1995). Northern slopes and valley bottoms in the area are occupied by Euro-Siberian species such as *Fagus sylvatica* L., *Corylus avellana* L., *Betula pendula* Roth, *Quercus pubescens* Mill. and *Quercus robur* L. The main bedrock type is calcareous conglomerate with abundant rocky outcrops distributed all over the area.

The climate of the region is characterized by a mean annual temperature of 12.8 ºC and a mean annual rainfall of around 1070 mm (climate data obtained from the Spanish meteorological station network for the period 1951-1999, AEMET, Agencia Estatal de Meteorología), corresponding to the temperate oceanic sub-Mediterranean bioclimatic region (Worldwide Bioclimatic Classification System, 1996-2009). In summer 2000, the study area experienced a severe drought episode with an average rainfall from June to August of 125 mm (Appendix 5.1). In August 2000, in addition to the rainfall scarcity and high temperatures, there was an intense regime of warm westerly winds (Agelet & Montserrat 2002). Although noticeable summer drought develops every year in the study area, climate records showed the absence of new extreme drought events since the 2000 drought episode until the sampling dates in November 2007 and March 2011, when the average rainfall from June to August was 205 and 295 (previous year 2010), respectively (see Appendix 5.1). In a preliminary analysis, the Catalan Forest Service associated the 2000 drought with increased canopy foliage dying and browning on around 20% of the study forest area (Agelet & Montserrat 2002). They distinguished four levels of canopy browning at the patch scale (see Figure 5.1 and Table 5.1): high (> 60% of holm oaks damaged), medium (30-60% of holm oaks damaged), low (< 30% of holm oaks damaged) and no canopy browning. High, medium and low canopy browning patches represented 42%, 40% and 18% of the damaged area, respectively.

Field sampling methods

In November 2007, 16 uneven-sized plots were surveyed along a transect using a nearest neighbor method based on the random pairs technique (Cottam & Curtis 1949). In this technique, distances among the nearest neighbors located at the same and the opposite site of transect are recorded to define the sampling area. Eleven plots were surveyed within the damaged stands previously delimited by the Catalan Forest Service (see Study site section and Figure 5.1: six plots within six different high canopy browning patches and five plots within five different low canopy browning patches), and five plots were surveyed within non-damaged, control areas. Distances between plots were always greater than 50 m, and sampled areas were selected so that they showed no signs of recent management or disturbances other than the studied drought. For all plots, total soil depth (measured with a metal stick from the beginning of the mineral soil to the bedrock with a precision of 1 cm) and topographic characteristics such as slope, aspect and altitude were recorded as correlates of water availability in the soil (Western *et al.* 2002).

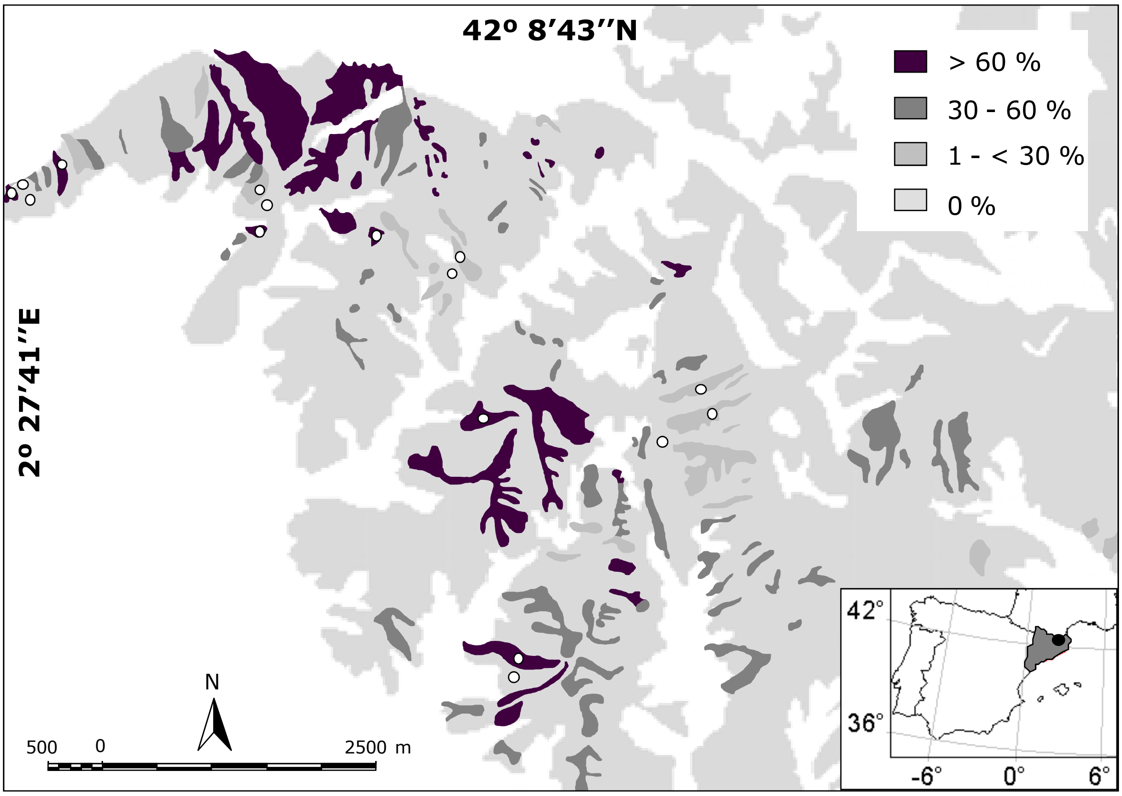

Figure 5.1. Location map of the study area showing sites of plots (white dots). Areas with high, medium, low and no canopy browning (see Material and Methods: Study site) are depicted with different grey shades within the distribution of holm oak in the area.

As a result of coppicing for charcoal production, holm oak trees usually exhibit a multi-stemmed structure. Accordingly, in each plot we sampled all stems (at least 2 cm diameter) of all adult individuals. For each stem, we considered four categories according to the crown condition: ND, no dieback or completely recovered; CS, branches' dieback and crown sprouters; LS, total crown mortality and lignotuber sprouters; D, total crown mortality without signs of resprouting (see Figure 5.2). For each tree, we measured diameter at breast height (DBH), basal area (from DBH), number of stems, height of the tallest stem, and we performed a whole characterization of crown condition at the tree level (ND, CS, LS or D categories as previously described for stems). In multi-stemmed individuals, tree crown condition was determined by the state of the size-dominant living stem (ND, CS or LS). A tree was considered dead (D) if all its stems were completely dry and without signs of resprouting. Note that due to the time lapsed since the drought episode this record does not allow assessing the immediate impact of the 2000 drought. We could not discern, for instance, whether ND stems had been damaged in 2000 and had recovered completely by 2007, or they simply had not been damaged. Stand density and total basal area were calculated at the plot level.

Table 5.1. Topographic and structural attributes of the studied forest at the patch and plot level, split by different levels of canopy browning.

	2000 Crown transparency			
	Control	< 30%	30-60%	> 60%
Local level		*N = 26*	*N = 63*	*N = 33*
Altitude (m)	–	644 (16)	641 (11)	682 (16)
Aspect (º)	–	193 (10)	188 (8)	163 (11)
Slope (º)	–	22 (1)	22 (1)	21 (1)
Plot level	*N = 5*	*N = 5*		*N = 6*
Stand density (ind/ha)	1647 (113)	1037 (77)	–	910 (192)
Basal area (m^2/ha)	34.6 (5.0)	21.7 (2.1)	–	17.5 (3.3)
Tree height (m)	7.2 (0.5)	5.9 (0.4)	–	4.8 (0.3)
Stems per tree	2.5 (0.3)	3.2 (0.4)	–	3.4 (0.2)
Soil depth (cm)	35.7 (1.2)	29.4 (2.7)	–	17.7 (1.4)
Dead individuals (D, %)	0.9 (0.9)	5.9 (3.7)	–	10.2 (5.6)
Crown sprouters (CS, %)	15.0 (6.3)	26.8 (3.7)	–	48.1 (4.6)
Lignotuber sprouters (LS, %)	0	0.9 (0.9)	–	15.5 (1.5)
Number of sampled trees	25	65	–	83

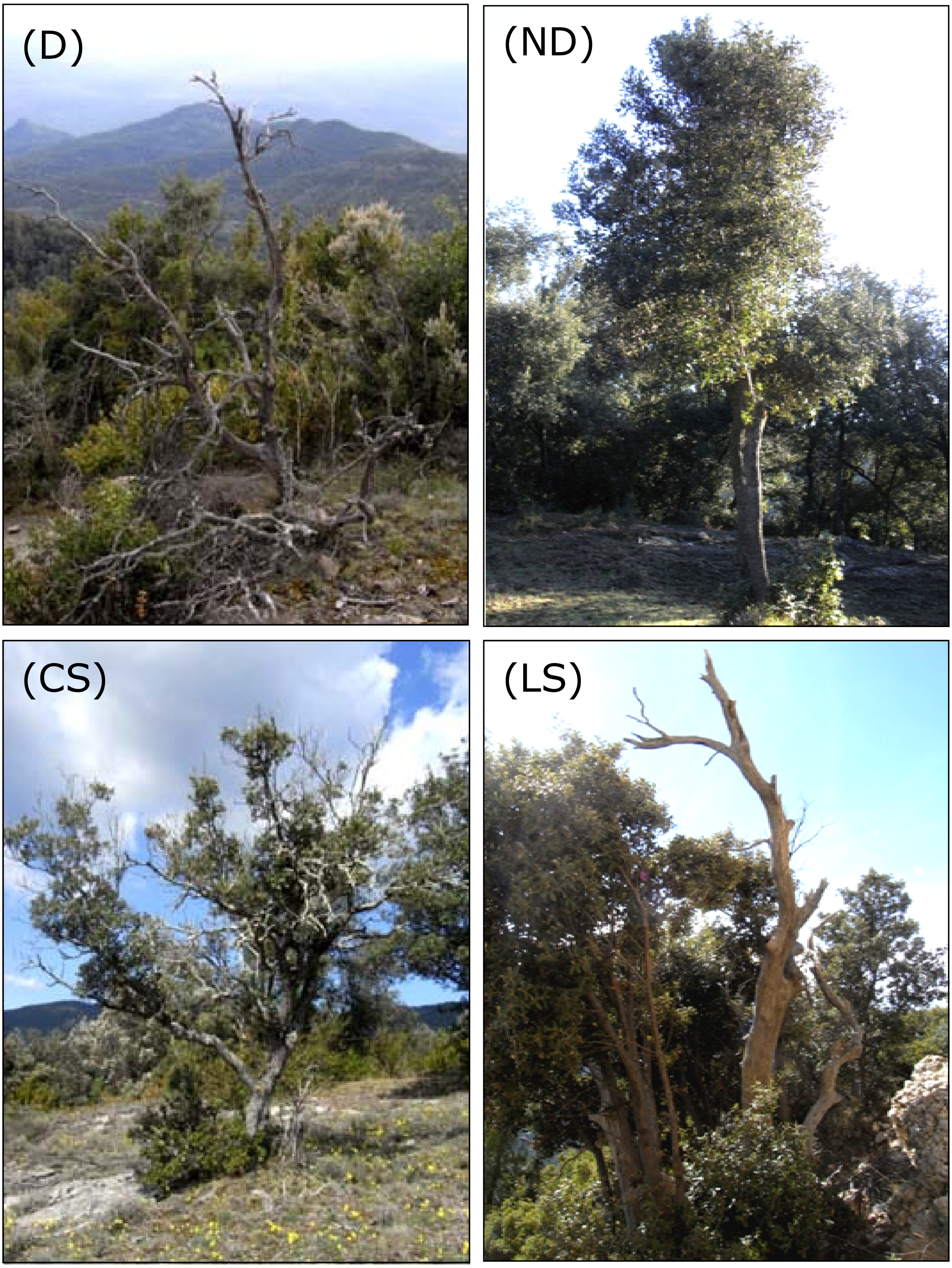

Figure 5.2. Photographs of dead (D) and living holm oak individuals, including three categories of crown condition: ND, no dieback or completely recovered; CS, branches' dieback and crown sprouters; LS, total crown mortality and lignotuber sprouters (see more details in Material and Methods: Field sampling methods).

For all plots, three to five individuals of each tree crown condition category (only living trees: ND, CS and LS) were excavated by digging around their lignotuber to a depth of 20 cm, obtaining approximately 1 cm thick samples of the external layer of the lignotuber. Previous assays with lugol solution indicated that most nonstructural carbohydrates (NSC) are stored in this external layer, which correspond to the sapwood area. These samples were used to analyze NSC and nutrients (see the "Lignotubers' nonstructural carbohydrates and nutrients" section). Overall, we sampled 173 individuals, with an unbalanced design because the CS and LS categories were scarce in low- and no-canopy browning patches. We also collected 20-30 exposed and apparently healthy current-year leaves from two mid-canopy branches of each sampled individual to analyze their carbon isotope composition and their nitrogen content (see the "Foliar carbon isotope and nitrogen content" section). Sampling of both lignotuber and leaves took place late at the end of the growing season (November) when pool reserves are expected to reflect the net balance between sinks and sources for the complete growing season, and have been shown to be relatively stable (Hoch *et al.* 2003; Sabaté *et al.* 1995). For the analysis of carbon isotope composition, leaves were collected on the same date to obtain integrated information of stomatal conductance and C assimilation for the whole growing season (Farquhar *et al.* 1989). In addition, in March 2011, tree crown condition was recorded again for all sampled individuals, except 15 individuals that could not be relocated.

Lignotubers' nonstructural carbohydrates and nutrients

Lignotuber samples were transported in a cooler over ice until sample processing in the laboratory on the same day. Once at the laboratory, the samples were microwaved for 90s to stop enzymatic activity, oven-dried for 72 h at 65 ºC, and grounded to fine powder. Nonstructural carbohydrates were defined as free sugars (glucose and fructose), low molecular weight sugars (free sugars and sucrose) plus starch, and were analyzed following the procedures described by Hoch *et al.* (2002), with some minor modifications. Approximately 12-14 mg of sapwood powder was extracted with 1.6 ml distilled water at 100 ºC for 60 min. After centrifugation, an aliquot of the extract was used for the determination of low molecular weight sugars after enzymatic (invertase from Saccharomyces cerevisiae and glucose hexokinase (GHK) assay reagent, I4504 and G3293 Sigma-Aldrich) conversion of sucrose and fructose into glucose. Another aliquot was incubated with an amyloglucosidase from *Aspergillus niger* (10115 Sigma-Aldrich) at 50 ºC overnight, to break down all NSC (starch included) to glucose. The concentration of free glucose was determined photometrically in a 96-well microplate reader (Sunrise^{TM} Basic Tecan, Männedorf, Switzerland) after enzymatic (glucose hexokinase (GHK) assay reagent) conversion of glucose to gluconat-6-phosphate. The dehydrogenation of glucose causes an increase in optical density at 340 nm. Starch was calculated as total NSC minus low molecular weight sugars. All NSC values are expressed as percent dry matter. Nutrient concentrations were analyzed at the Chemistry Laboratory (SAQ) at the Autonomous University of Barcelona, by combustion gas chromatography using a CHNS-O Elemental Analyzer Euro EA3000 (EuroVector SpA, Milan, Italy) for determining total nitrogen, and by Inductive Coupled Plasma (ICP-OES) Spectrometry Optima 4300 analysis (PerkinElmer Inc., MA, USA) after pressure digestion with HNO_3 for determining total phosphorus.

Foliar carbon isotope and nitrogen content

Current-year leaves were oven-dried for 72 h at 65ºC and ground to fine podwer. Ground samples were analyzed for carbon stable isotope composition and nitrogen content at the Cornell Isotope Laboratory (COIL) at Cornell University, using a Thermo Delta V isotope ratio mass spectrometer (IRMS) interfaced to a NC2500 elemental analyzer. The carbon stable isotope composition was expressed in delta notation: $\delta^{13}C$ (‰) = ($R_{sample}/R_{standard}$ – 1) x 1000, where R_{sample} is the $^{13}C/^{12}C$ ratio of the sample and $R_{standard}$ is the $^{13}C/^{12}C$ ratio of the international Vienna Pee Dee Belemnite carbon standard. Precision (standard deviation = 0.10‰) and accuracy (0.05‰) were determined by analysis of an in-house isotope standard ("BCBG"), which is calibrated against IAEA standards. Nitrogen concentrations are expressed as percent dry matter.

Statistical analyses

Generalized linear mixed models were used to study the environmental correlates of tree mortality, stem mortality and tree crown condition at the individual level (N = 368) with plot effects modeled as a random factor to account for the spatial autocorrelation among individuals within plots. Tree mortality was binomially distributed. For stem mortality and tree crown condition, the number of standing dead stems and the number of damaged stems (crown condition CS, LS or D) in each tree were considered as count response variables. The logarithm of the total number of stems per tree was introduced into both models as an explanatory variable, and errors were assumed to follow a Poisson distribution which is effectively similar to (but more flexible than) modeling the proportion of dead or damaged stems per tree (Faraway 2006). We conducted additional analyses using a more restrictive definition of damaged tree crown condition considering only those trees exhibiting stems with total crown mortality (D or LS categories), obtaining very similar results (not shown).

All models (tree mortality, stem mortality and tree crown condition) were identical in terms of predictor variables and included: tree basal area (ln transformed), stems per tree (ln transformed), plot basal area, plot soil depth, plot aspect, and plot altitude. All predictor variables were normally distributed or could be normalized using standard transformations except stems per tree, which remained slightly normal after logarithmic transformation. Aspect was normally distributed, as the measured variable covered a relatively narrow range between 150 and 250º across all plots. We included the interaction between ln(stems per tree) and plot basal area because competition between stems within the same individual may be influenced by competition at the stand level, and the interaction between plot basal area and plot soil depth because competition at the stand level may be more intense on shallow soils. Some variables were not introduced into the models because they were highly correlated to other predictor variables: tree height was correlated to plot soil depth (*r* = 0.801, *P* < 0.001), and plot density of individuals was correlated to plot basal area (*r* = 0.872, *P* < 0.001). The residuals of all models were normally distributed. A Moran's test (Cliff and Ord 1981) of the residuals indicated that it was not necessary to further correct for the spatial structure of the data. Parameters (β) of all fitted models were estimated using maximum likelihood methods, and model selection was performed using the Akaike's information criterion (AIC).

Additional analyses were carried out using analysis of variance (One-Way ANOVA using Post Hoc Multiple Comparisons) to compare the amounts of carbon and nutrient reserves stored in lignotubers and the physiological performance of leaves among trees with different crown condition. The relationship between NSC stored in lignotubers in 2007 and changes in crown condition of trees between 2007 and 2011 was analyzed using Two-Way ANOVA tests, with tree crown condition in 2007 (ND, CS or LS) and the qualitative change of tree crown condition between 2007 and 2011 (improved, equal or worsened) as the independent factors. Note that an improvement for CS and LS categories implies that new resprouts surpass the initial height of the tree. By contrast, a worsened change implies that ND individuals become CS, LS or D, CS individuals become LS or D, and LS individuals become D. We also used Pearson and Spearman correlation coefficients as a measure of association between pairs of variables. All statistical analyses were carried out with R version 2.12.0 (R Development Core Team, 2010).

RESULTS

Determinants of crown condition

The proportion of trees exhibiting some degree of prolonged crown damage (CS, LS or D individuals) varied with the level of canopy browning in the stand, being 15.9%, 33.6% and 73.8% in no, low and high canopy browning patches, respectively (Table 5.1; see also Figure 5.1). At the tree level, mortality was mostly influenced by the number of stems per tree and, to a lesser extent, by tree basal area: larger number of stems per tree and higher basal area were associated to higher probabilities to survive (Table 5.2a). Some plot attributes, such as soil depth and altitude, were also significant in the mortality model. Increases in soil depth were associated to higher survival probabilities, while location at higher altitude was associated with higher mortality. Interactions among explanatory variables were not significant and were removed from the final model because including them worsened model fit in terms of AIC.

Table 5.2. Generalized linear mixed models for tree mortality (a), stem mortality (b) and tree crown condition (c) at the individual level (N = 368).

(a) Tree mortality	β	SE	z	P
Intercept	-11.737	6.369	-1.843	0.065
Ln[Tree basal area (cm^2)]	-0.659	0.305	-2.156	0.031
Ln[Stems per tree]	-1.387	0.525	-2.644	0.008
Plot basal area (m^2/ha)	0.003	0.045	0.074	0.940
Plot soil depth (cm)	-0.172	0.060	-2.851	0.004
Plot aspect (º)	0.022	0.014	1.531	0.126
Plot altitude (m)	0.018	0.007	2.621	0.008

(a) Stem mortality	β	SE	z	P
Intercept	-5.853	2.909	-2.012	0.044
Ln[Tree basal area (cm^2)]	-0.399	0.117	-3.390	<0.001
Ln[Stems per tree]	0.727	0.301	2.415	0.015
Plot basal area (m^2/ha)	-0.013	0.026	-0.501	0.616
Plot soil depth (cm)	-0.090	0.026	-3.493	<0.001
Plot aspect (º)	0.014	0.006	2.470	0.013
Plot altitude (m)	0.007	0.003	2.070	0.038
Ln[Stems per tree] * plot basal area	0.030	0.012	2.471	0.013

(a) Tree crown condition	β	SE	z	P
Intercept	-5.212	2.939	-1.773	0.076
Ln[Tree basal area (cm^2)]	-0.087	0.073	-1.188	0.235
Ln[Stems per tree]	1.063	0.097	10.872	<0.001
Plot basal area (m^2/ha)	0.007	0.020	0.364	0.715
Plot soil depth (cm)	-0.096	0.026	-3.619	<0.001
Plot aspect (º)	0.013	0.006	2.101	0.035
Plot altitude (m)	0.006	0.003	1.681	0.092

The number of damaged stems (tree crown condition model; Table 5.2c) and the number of dead stems (stem mortality model; Table 5.2b) were associated negatively to soil depth and positively to aspect (higher damage was found on western aspects). Location at higher altitudes and smaller tree basal areas were also associated to higher mortality of stems. The number of damaged stems increased proportionally with the number of stems per tree ($\beta \approx 1$, Table 5.2c). Interestingly, in the stem mortality model, the interaction between the number of stems per tree and plot basal area was significant, indicating that the effect of the total number of stems on stem mortality becomes larger as plot basal area increases (from $\beta \approx 1$ at the lowest value of plot basal area, to $\beta \approx 2.5$ at the highest basal area; see Table 5.2b and Appendix 5.2).

Carbon and nutrient reserves

The most damaged living trees (LS) presented lower current water-use efficiencies estimated from foliar δ^{13}C than trees resprouting from the crown (CS), while control trees with no dieback (ND) showed intermediate values (Table 5.3 and Figure 5.3a). Leaf N content did not differ among the three crown condition categories (Table 5.3 and Figure 5.3b) and it was unrelated to leaf δ^{13}C (r = 0.026, P = 0.731).

NSC concentrations stored in lignotubers were depleted by 26% and 60% in CS and LS individuals, respectively, relatively to healthy (ND) trees, which were considered as controls (Table 5.3 and Figure 5.4a). Basal area of trees was not related to NSC concentrations (r = 0.091, P = 0.233) and, thus, differences in NSC concentrations among the three categories of crown condition were not caused by differences in tree basal area. Interestingly, the amount of carbon reserves analyzed in 2007 was positively associated to the improvement of crown condition between years 2007 and 2011 (Two-Way ANOVA F = 14.845, d.f. = 2, P< 0.001; Figure 5.5). Regarding the nutrients stored in lignotubers, contrary to the case of NSC concentrations, LS individuals contained larger amounts of N and P than ND and CS individuals, although significant differences only occur between CS and LS individuals for both nutrients (Table 5.3 and Figure 5.4b and 5.4c).

Table 5.3. One-Way ANOVA tests of the effects of tree crown condition (ND, no dieback or completely recovered; CS, branches' dieback and crown sprouters; LS, total crown mortality and lignotuber sprouters; see more details in Material and Methods: Field sampling methods) on carbon isotopic composition and N concentration of leaves and the amounts of carbon and nutrient reserves (N and P) stored in lignotubers.

		Leaf δ^{13}C (‰)		Leaf N (%)		Lignotuber NSC (%)		Lignotuber N (%)		Lignotuber P (%)	
	d.f.	F	P	F	P	F	P	F	P	F	P
Crown condition	2, 170	5.378	0.005	1.491	0.228	65.915	<0.001	3.572	0.03	4.574	0.012

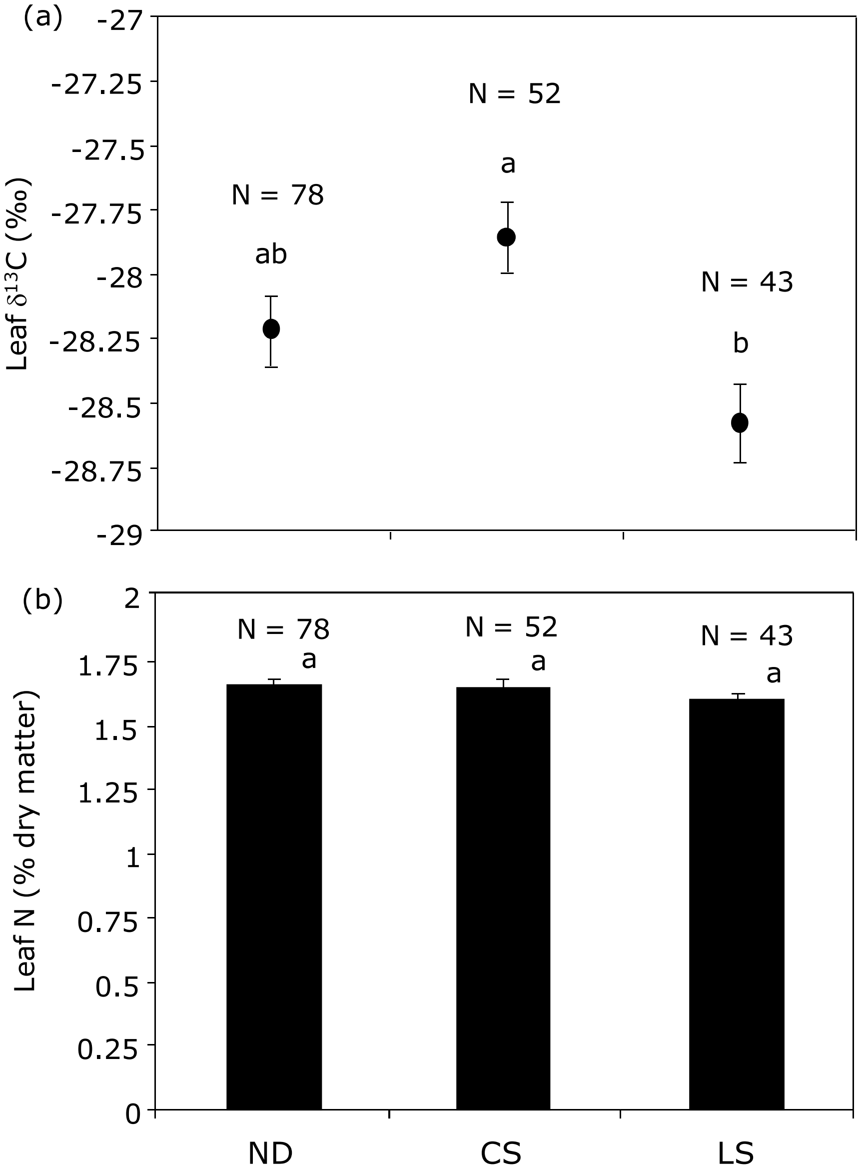

Figure 5.3. Leaf $\delta^{13}C$ (a) and leaf N (b) measured in 2007 as a function of tree crown condition: ND, no dieback or completely recovered; CS, branches' dieback and crown sprouters; LS, total crown mortality and lignotuber sprouters (see more details in Material and Methods: Field sampling methods). Different letters indicate statistically significant differences (One-Way ANOVA test using Post Hoc Multiple Comparisons). Error bars show standard errors. The number of trees (N) for each group is also shown.

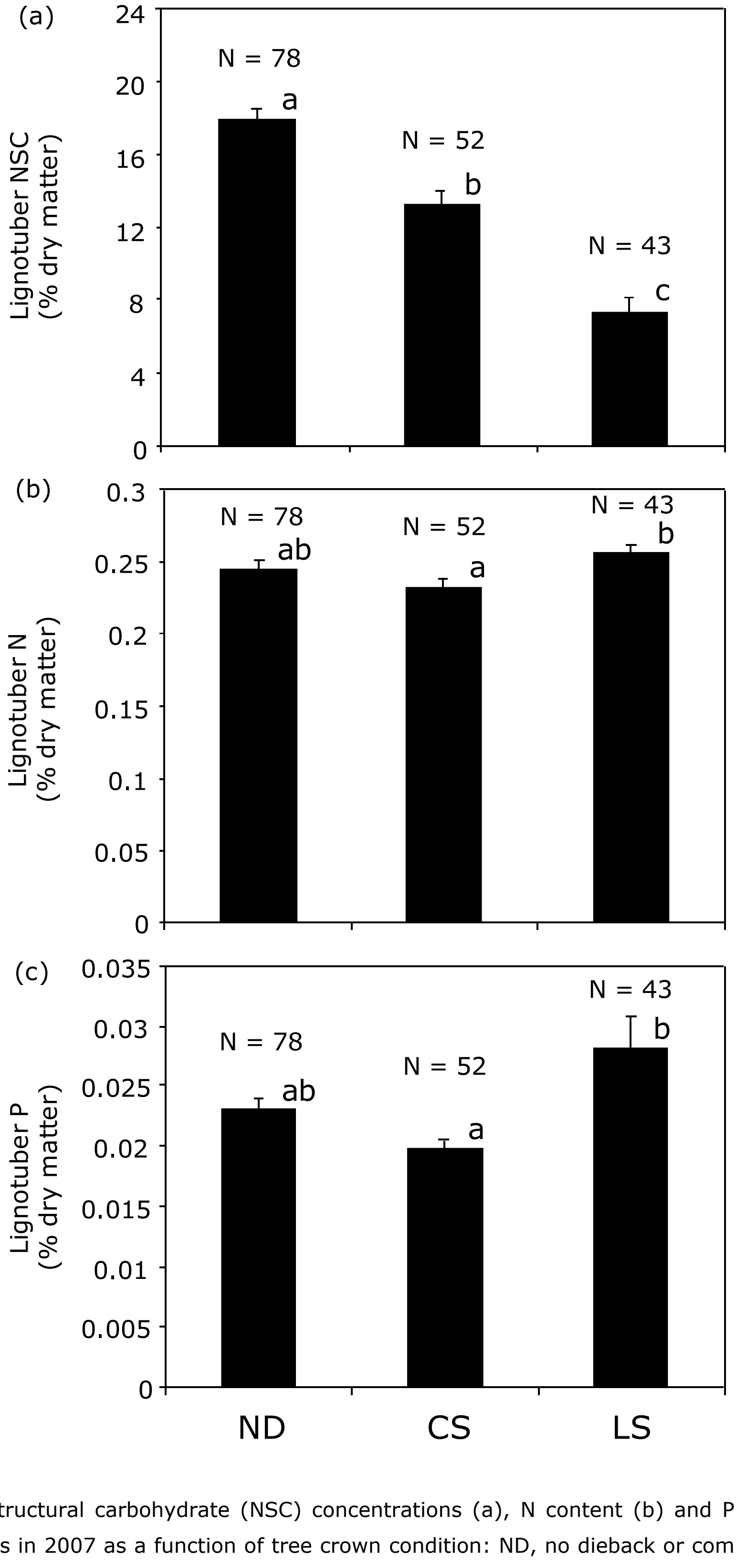

Figure 5.4. Nonstructural carbohydrate (NSC) concentrations (a), N content (b) and P content (c) stored in lignotubers of trees in 2007 as a function of tree crown condition: ND, no dieback or completely recovered; CS, branches' dieback and crown sprouters; LS, total crown mortality and lignotuber sprouters (see more details in Material and Methods: Field sampling methods). Different letters indicate statistically significant differences (One-Way ANOVA test using Post Hoc Multiple Comparisons). Error bars show standard errors. The number of trees (N) for each group is also shown.

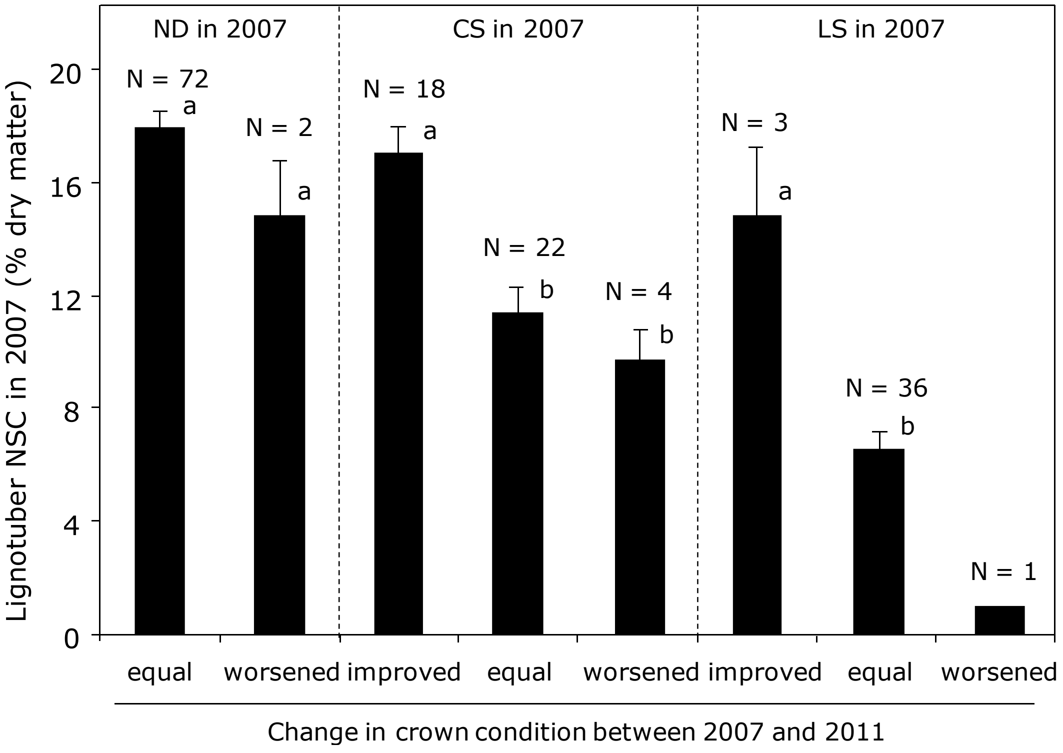

Figure 5.5. Nonstructural carbohydrate (NSC) concentrations stored in lignotubers of trees in 2007 as a function of the qualitative change of crown condition occurred in trees between 2007 and 2010 (improved, equal or worsened; see Material and Methods: Statistical analyses). Broken lines segregate groups of trees according to the crown condition in 2007 (ND, CS or LS; see more details in Material and Methods: Field sampling methods). Different letters indicate statistically significant differences between groups of trees with different qualitative change of crown condition within the previous groups (Two-Way ANOVA test using Post Hoc Multiple Comparisons). Error bars show standard errors. The number of trees (N) for each group is also shown.

DISCUSSION

Determinants of crown condition

We report prolonged effects of an extreme drought episode (cf. Bréda & Badeau 2008). Seven years after the drought, up to 73.8% of trees still showed some level of crown damage in some stands as a result of the stem and branch mortality induced during the past drought. The patchy pattern of damage observed in the studied area suggests that a wide range of other causes operating at local or micro-local scale (for instance, microtopography or stand structure likely related to past management) are involved in predisposing trees to be more susceptible to climatic drought (Manion 1991; see also Bigler *et al.* 2006 and Galiano *et al.* 2010). Contrasting drought effects are explained in our case by geological substrate and, particularly, by total soil depth (similar to Lloret *et al.* 2004). Although precise records of soil water availability are not available, soil depth is particularly relevant controlling water availability at the plot level in the studied forests, with shallow soils that develop over conglomerates. This bedrock impedes roots growth into deep layers (Lloret *et al.* 2004) and provides low water supply to plants, particularly under low-rainfall conditions (cf. Peñuelas *et al.* 2000, also for *Quercus ilex*; see also López-Soria & Castell 1992). Nevertheless, location at higher altitudes also determined the ultimate mortality of trees in our case (Table 2). In the study area holm oak forests occupy a relatively narrow range of altitudes, and the effect of exposure to wind and direct solar radiation on the ridge tops apparently prevails over the effect of cooler temperatures at higher altitudes.

Mortality tends to be higher in small-sized individuals (smaller basal areas), likely due to their more superficial rooting system and to their smaller bud bank and resprouting ability (Trabaud 1987; Obón 1997; Pugnaire *et al.* 2000; cf. Lloret *et al.* 2004, also for *Quercus ilex*). By contrast, increasing number of stems per tree implies higher drought survival. In general, disturbances trigger the growth of the meristematic tissue of lignotubers, which are further enlarged by the fusion of the stem bases of the new emerging shoots (Canadell *et al.* 1999). Enlarged lignotubers provide large amounts of resources that will ensure at least the survival of one of the stems and, thus, the perpetuation of the individual.

Water availability per unit of basal area is normally lower in areas with higher basal area (cf. Callaway & Walker 1997; Briones *et al.* 1998). The relatively high stem density in our particular holm oak forest is due to the reduction of coppicing since the late 1970s (Agelet & Montserrat 2002), as has been also observed in other areas (Barbéro *et al.* 1992; Terradas 1999). In our case, competition at stand level seems not to be intensified by lower soil water availability, as suggested by the absence of a significant interaction between the effects of plot basal area and soil depth. However, competition among stems within the same individual appears to be influenced by the total basal area of the stand, as has long been observed in other holm oak forests (Retana *et al.* 1992; Gracia *et al.* 1999). In these forests, intense competition normally causes very low growth rates that may lead to an almost permanent state of stagnation (Gracia *et al.* 1999). Extreme dry conditions occurred in summer 2000 would have triggered self-thinning within individuals that resulted in a release of competition. Our results suggest that thinning could reduce competition and improve the resistance of holm oak forests to climate change-type droughts (see also Cotillas *et al.* 2009; Sánchez-Humanes & Espelta 2011; Rodríguez-Calcerrada *et al.* 2011), although the relationship between short-term stimulation of growth induced by thinning (López-Soria & Castell 1992; Retana *et al.* 1992) and carbon storage merits further investigation (cf. López *et al.* 2009).

Carbon and nutrient reserves

We found that individuals resprouting from lignotuber exhibited lower water-use efficiency and higher levels of nutrient reserves compared to those individuals that maintain a living crown (cf. Castell *et al.* 1994, also for *Quercus ilex*). Resprouting individuals normally rely upon their preexisting root system, which results in a greater root-to-shoot ratio and higher availability of soil resources per unit of leaf area (Savé *et al.* 1999).

Stored carbohydrates are the most important sources supporting new growth after disturbances that result in seriously reduced leaf area and carbon assimilation (Kays & Canham 1991; Canadell & López-Soria 1998; López *et al.* 2009). To our knowledge, this is the first study examining the replenishment of carbon reserves in resprouter trees after a natural drought episode. Other studies have documented return times of 20 years to completely recover carbon reserves in lignotubers of holm oaks after severe experimental thinning (López *et al.* 2009). In our case, stored carbon reserves were still depleted by 60% in highly drought-damaged trees seven years after the episode. In contrast, nutrient pools are completely recovered seven years after the drought, assuming some degree of depletion during tree resprouting after drought, as reported by previous studies on disturbed Mediterranean woody plants (cf. Canadell & López-Soria 1998, for *Arbutus unedo* and *Erica arborea*). It should be noted, however, that in our study measures of carbohydrates storage were more representative than those of nutrients, as the latter include both available and structural forms.

Successive surveys in 2007 and 2011 showed a direct association between carbon reserve depletion and further deterioration of crown condition, as has been shown by earlier studies (Bréda *et al.* 2006). Our interpretation is that current levels of carbon uptake still suffer from a reduced photosynthetic area and they are not enough to meet the demands – new growth, respiration – of an extensive root system and the replenishment of pool reserves (Canadell & López-Soria 1998). Low levels of stored carbohydrates have been associated to increased risk to suffer drought-induced mortality (McDowell *et al.* 2008; Galiano *et al.* 2011; see also Sala *et al.* 2010; McDowell *et al.* 2011). Our results highlight the determinant role of carbon reserves on tree-level responses to drought and subsequent recovery, and suggest that an increase in the frequency of dry periods, as may occur according to climate change projections (IPCC 2007), may lead trees to a progressive depletion of carbon reserves and, consequently, to a loss of resilience in face of future droughts.

In conclusion, our study documents a drought-driven forest dieback episode in which soil depth and characteristics of individual trees determined tree-level effects, illustrating how climatic trends interact with local drivers to produce complex patterns of responses at the landscape level. We show how this dieback results in a depletion of carbon reserves that lasts for several years after the episode, which, in turn, is likely to induce a progressive loss of forest resilience in face of future droughts. Under climate change conditions, holm oak forests may be particularly challenged if drought events become more frequent and intense and return periods become shorter than the time period required for reserves to recover.

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Capítulo 6

Discusión general y conclusiones

Los fenómenos de decaimiento del bosque asociados a los cambios recientes en el clima son objeto de creciente interés, entre otros motivos porque parecen estar comprometiendo el papel de los bosques como sumideros de carbono a nivel global, reduciendo su producción primaria neta y convirtiéndolos en fuentes de carbono (Clark 2004; Kurz *et al.* 2008; Zhao & Running 2010). Esta tesis documenta y analiza dos episodios de mortalidad en bosques inducida por sequía en la Península Ibérica, contribuyendo al creciente número de casos documentados en los últimos años en comunidades forestales de todo el mundo (ver revisión en Allen *et al.* 2010). La aportación de nuevos casos es importante porque ayuda a valorar la dimensión del problema, y a esclarecer cuáles son los mecanismos que hacen que ciertos ecosistemas o biomas sean más sensibles que otros a los eventos climáticos extremos (cf. Martínez-Vilalta *et al.* 2011). En este sentido, estudios recientes apuntan a la importancia de analizar también aquellos episodios de sequía extrema que no provocan respuestas en la vegetación (Smith 2011; Lloret *et al.* 2012). Las principales aportaciones de esta tesis se pueden discutir estructuradas en cuatro apartados. Uno respondiendo a cuáles son los factores determinantes de la defoliación y la mortalidad en condiciones de sequía extrema; otro analizando el papel del uso y almacenamiento de los recursos en la recuperación posterior a la sequía; otro examinando las implicaciones de los resultados anteriores en relación a los mecanismos fisiológicos que subyacen a la muerte de los árboles; y un último analizando los cambios en los patrones de reclutamiento en respuesta a la sequía. Estas cuestiones se han ido abordando en los capítulos de la tesis en un orden ligeramente distinto. Los capítulos 2 y 3 analizan los factores que determinan los patrones de defoliación y mortalidad en un bosque de pino albar (*Pinus sylvestris* L.) afectado por un episodio reciente de sequía extrema, así como los factores que influyen sobre los patrones de reclutamiento de especies de *Quercus* que potencialmente podrían reemplazar al pino albar. El capítulo 4 evalúa el estado de recuperación del bosque de pino albar cuatro años después de la sequía, haciendo especial hincapié en los niveles de reservas de carbono y la defoliación de las copas como factores clave en la recuperación y la supervivencia de los árboles. Y finalmente, en el capítulo 5 se analizan los patrones de afectación de las copas de una población de encina (*Quercus ilex* L.) siete años después de un episodio de sequía extrema, y su relación con las reservas de carbono y nutrientes almacenadas en el lignotuber.

Factores determinantes de la defoliación y la mortalidad

Los resultados de esta tesis demuestran la naturaleza multifactorial del fenómeno del declive del bosque inducido por sequía en los dos episodios estudiados. En ambos casos los patrones de defoliación y mortalidad observados sugieren que un amplio rango de causas actúan como factores de predisposición a escala local o micro-local, haciendo que los árboles sean más susceptibles a las sequías climáticas (Waring 1987; Franklin *et al.* 1987; Manion 1991; ver también Bigler *et al.* 2006). El caso estudiado del pino albar es uno de los pocos que ha explorado exhaustivamente la mayoría de los factores extrínsecos e intrínsecos del bosque que potencialmente podrían estar asociados con la muerte de los árboles. Los resultados muestran que estos factores actúan de manera distinta y jerárquica sobre los parámetros de declive: mientras que en la defoliación intervenían un número importante de factores (déficit hídrico climático estimado para cada parcela, estructura del rodal, propiedades del suelo, infección por muérdago), los factores que acababan contribuyendo significativamente a la muerte del árbol quedaban reducidos al déficit hídrico climático y a la estructura del rodal. Es particularmente relevante el papel de la estructura del bosque en la muerte de los pinos, entre otros motivos por ser un factor sobre el que se puede incidir mediante la gestión de los bosques. En el caso de la encina, la estructura del bosque, la topografía y la profundidad del suelo se asociaron a los efectos prolongados de la sequía sobre los árboles, siendo en particular la altitud el factor más asociado con la muerte durante el episodio de sequía. Esto se debe a que este bosque de encina en particular ocupa un rango relativamente estrecho de altitudes, de manera que el efecto de la exposición al viento y a la radiación solar directa en las crestas prevalece sobre el efecto de las bajas temperaturas. Una de las conclusiones es que puesto que los efectos de la sequía sobre los bosques responden a un patrón multifactorial, en cada caso concreto las particularidades de los factores locales merecen ser examinadas de cara a realizar actuaciones de gestión con el objeto de paliar los efectos de las sequías extremas.

Pocos estudios han explorado el papel de las propiedades del suelo en los procesos de mortalidad en bosques inducida por sequía. En los dos episodios de mortalidad estudiados, la profundidad del suelo se asociaba de manera importante con los patrones de defoliación y mortalidad, siendo los suelos menos profundos aquellos donde los efectos de la sequía fueron mayores (ver Lloret *et al.* 2004). En el caso de la encina, además, los suelos poco profundos se desarrollaban sobre sustratos de conglomerados. Este sustrato rocoso impide el crecimiento de las raíces hacia capas más profundas del suelo (Lloret *et al.* 2004), y proporciona un bajo suministro de agua a las plantas, particularmente en condiciones de baja precipitación (cf. Peñuelas *et al.* 2000; ver también López-Soria & Castell 1992). En el caso del pino albar, los resultados demostraron que las zonas menos afectadas se encontraban distribuidas sobre suelos más arcillosos. Los suelos arcillosos retienen más agua a un cierto nivel de potencial hídrico y, por lo tanto, causan una disminución más gradual de la humedad del suelo a medida que el contenido de agua disminuye (Sperry *et al.* 1998).

En relación a las características estructurales del bosque, la disponibilidad hídrica por unidad de área basal es normalmente inferior en zonas con elevadas áreas basales (cf. Callaway & Walker 1997; Briones *et al.* 1998). En las últimas décadas, los dos bosques estudiados han experimentado un aumento relativo en su densidad de individuos y/o pies, y esto se debe fundamentalmente al abandono del manejo forestal que han sufrido estos bosques, y que también se ha venido observando de manera generalizada en la Península Ibérica (Barbéro *et al.* 1992; Terradas 1999; Poyatos *et al.* 2003; Linares *et al.* 2009). En el caso del pino albar, sin embargo, la ausencia de interacción significativa entre los efectos de la sequía y de la estructura del bosque sugiere que ambos factores ejercen efectos aditivos sobre el bosque estudiado. Otros estudios, sin embargo, han encontrado mayores efectos de sequías climáticas en los bosques más densos de pino albar a escala de la Península Ibérica (Vilà-Cabrera *et al.* 2011). En nuestro caso, los efectos del episodio de sequía severa del año 2004-2005 sucedieron sobre un proceso natural de auto-tala desencadenado a partir de los cambios en el manejo del bosque. En el caso de la encina, las características edáficas de la zona provocaban que las zonas más afectadas por la sequía fueran aquellas con una menor profundidad del suelo, y con menos árboles. Sin embargo, estos árboles presumiblemente experimentaban una elevada competencia entre el gran número de pies por individuo surgidos como resultado del cese de las prácticas de resalveo en los años 70 (Agelet & Montserrat 2002). En este tipo de bosques, la intensa competencia entre pies y entre individuos normalmente causa tasas de crecimiento muy bajas que pueden conducir al bosque a estados semi-permanentes de estancamiento (Gracia *et al.* 1999). Las condiciones de extrema sequía en el año 2000 seguramente desencadenaron procesos de auto-tala de algunos pies dentro de individuos que resultarían en la liberación de competencia entre los pies supervivientes.

La presencia de plantas hemiparásitas se vio asociada a los patrones de defoliación observados en la población de pino albar. La defoliación de los pinos aumentaba progresivamente cuánto más intensa era su infección por muérdago (ver Dobbertin & Rigling 2006). Esto es consistente con el hecho de que cuando los árboles reducen sus tasas de transpiración cerrando los estomas en condiciones de estrés hídrico, el muérdago continúa transpirando e incrementa, así, el estrés hídrico de los árboles huésped (Fischer 1983; Press *et al.* 1988; Strong & Bannister 2002). El papel del muérdago puede ser significativo también en la economía de otros recursos, como los nutrientes. Este hecho ha quedado contrastado en este estudio al observarse que, la infección por muérdago reducía el contenido de nitrógeno en las hojas, disminuyendo la asimilación de carbono y afectando negativamente el crecimiento. Aunque no se pudo descartar que los árboles más defoliados fueran más vulnerables a sufrir nuevas infecciones por muérdago, la avanzada edad de la mayor parte de plantas de muérdago sugería claramente que dicha infección era anterior al episodio de sequía, y que probablemente el muérdago actuó como un factor de predisposición durante el episodio de sequía del 2004-2005. Los árboles más grandes presentaban intensidades de infección por muérdago más elevadas, probablemente porque las grandes ramas proporcionan posaderos de alimentación a los pájaros dispersores de las semillas de muérdago (Aukema & Martínez del Rio 2002). No obstante, bajo las mismas condiciones de intensidad de infección, los árboles pequeños presentaban mayores defoliaciones. Los árboles pequeños normalmente poseen raíces menos profundas, lo que les hace menos competitivos por los recursos hídricos subterráneos (Pugnaire *et al*. 2000). Múltiples estudios han documentado la mayor vulnerabilidad de los árboles pequeños a sufrir efectos en condiciones de sequía (Martínez-Vilalta & Piñol 2002; Lloret *et al.* 2004; Smith *et al.* 2005; Bravo-Oviedo *et al.* 2005; Chen *et al.* 2008), incluido también el caso de la encina estudiado en esta tesis.

Factores determinantes de la recuperación posterior a la sequía

Las dos poblaciones estudiadas en esta tesis aun se estaban recuperando de los efectos de la sequía varios años después de los episodios correspondientes en cada caso. A pesar de la capacidad rebrotadora de la encina tras sufrir una perturbación, el 10% de encinas muertas en las zonas más afectadas es relativamente comparable al 14% de individuos muertos encontrados en la población de pino albar. De hecho, siete años después de la sequía, el 74% de encinas aun mostraban daños en las copas frente al 60% de los pinos que mostraban más del 50% de defoliación dos años después del episodio de sequía en la población de pino albar. No obstante, aunque es interesante contrastar los patrones de recuperación después de episodios de sequía extrema de dos especies con estrategias de recuperación distintas, estos resultados no son directamente comparables puesto que las condiciones de sequía y las características de cada localidad son distintas. En ambas poblaciones, se ha podido demostrar que los niveles de reservas de carbono almacenados en el tronco o el lignotuber estaban relacionados con el estado de recuperación que mostraban los árboles. Los árboles con un peor estado de recuperación mostraban una reducción importante de reservas, lo que supone una pérdida de resiliencia de cara a afrontar nuevos episodios de sequía.

En el caso del pino albar, los árboles que habían recuperado menos del 50% del follaje verde cuatro años después del episodio de sequía contenían niveles muy bajos de reservas de carbono. Además, tanto el follaje verde como los niveles de carbono estaban relacionados con el impacto que tuvo la sequía sobre el crecimiento radial, y a su vez ambas variables estaban mutuamente correlacionadas. Sin embargo, nuestro modelo más probable mostraba que el agotamiento actual de las reservas de carbono es el resultado de la reducción en la absorción fotosintética de carbono, consecuencia tanto de la reducción del área foliar como del cierre estomático. La escasez de agua en condiciones de sequía provoca la reducción de la conductancia estomática, el área foliar, el crecimiento radial, y la producción de yemas (Tyree *et al.* 1993; Bréda *et al.* 2006; Pichler & Oberhuber 2007). El daño en las yemas puede limitar la capacidad de los árboles de crear nuevo tejido fotosintético y nuevas ramas durante los años posteriores a la sequía (Power 1994; Stribley & Ashmore 2002). No obstante, la reducción del área foliar es un mecanismo de aclimatación a la escasez de agua bien conocido en el pino albar (Martínez-Vilalta *et al.* 2009), y la consecuente limitación en la absorción de carbono se ha asociado con reducciones en el crecimiento también en otros pinos (por ejemplo, Borghetti *et al.* 1998; Sterck *et al.* en prensa). En cualquier caso, la relación positiva entre la cantidad de hojas y los niveles de reservas de carbono constituye un potencial mecanismo de retroalimentación positiva que limita la recuperación de los árboles (menos hojas implica menos reservas de carbono y viceversa). Este mecanismo explicaría los efectos prolongados de la sequía observados en múltiples estudios (Peñuelas *et al.* 2001; Lloret *et al.* 2004; Bréda & Badeau 2008), así como los largos periodos de decaimiento registrados antes de la muerte en diversas especies, incluido el pino albar (Bigler *et al.* 2006; Hereş *et al.* 2011).

En el caso de la encina, el estado de las copas se asociaba con el nivel de reservas de carbono almacenadas en el lignotuber siete años después del episodio de sequía. Los carbohidratos almacenados representan la fuente más importante de recursos que sustenta el nuevo crecimiento tras las perturbaciones que suponen una reducción drástica del área foliar y la asimilación de carbono (Kays & Canham 1991; Canadell & López-Soria 1998; López *et al.* 2009). Hasta donde sabemos, este es el primer estudio que examina la reposición de las reservas de carbono en una especie rebrotadora de árbol tras un episodio de sequía en condiciones naturales. Otros estudios han documentado en la encina tiempos de retorno de hasta 20 años para recuperar completamente las reservas de carbono almacenadas en el lignotuber después de talas experimentales (López *et al.* 2009). En nuestro caso, en aquellos árboles más severamente dañados las reservas de carbono en el lignotuber aún eran un 60% menores siete años después del episodio de sequía. La asimilación de carbono por parte de la reducida área foliar no es suficiente para cubrir las demandas de nuevo crecimiento, respiración de un sistema de raíces extenso y reposición de las reservas de carbono. Por el contrario, las reservas de nutrientes estaban completamente recuperadas siete años después del evento, e incluso eran mayores en los árboles más afectados. Este hecho puede explicarse porque los individuos que sufrieron muerte total de la copa y rebrotaron desde el lignotuber dependen del sistema de raíces formado antes de la sequía, de manera que presentan ratios de raíces vs. tallos elevadas y una gran disponibilidad de recursos del suelo por unidad de área de hoja (Savé *et al.* 1999).

Implicaciones en los mecanismos fisiológicos de mortalidad inducida por sequía

Los resultados de esta tesis destacan el importante papel que tienen las reservas de carbono sobre la supervivencia y la recuperación de los individuos después de sufrir los efectos de una sequía extrema. Tanto en el caso del pino albar como de la encina, las reservas de carbono estuvieron muy relacionadas con el estado de recuperación que presentaban los árboles unos años después del episodio. Además, aquellos árboles con un nivel de reservas extremadamente bajo sufrieron un deterioro adicional varios años después del evento, llegando incluso a producirse la muerte de algunos individuos. Estos resultados se obtuvieron de manera más clara para el caso del pino albar. Por tanto, los resultados de esta tesis muestran por primera vez una asociación directa entre el agotamiento de las reservas de carbono y la muerte del árbol asociada a un episodio de sequía (cf. McDowell *et al.* 2008). Hasta la obtención de estos resultados, las evidencias a favor de la hipótesis del agotamiento de reservas eran indirectas (Adams *et al.* 2009; Breshears *et al.* 2009) y consistentes con interpretaciones alternativas (Sala 2009; Leuzinger *et al.* 2009). Aunque no son concluyentes respecto al mecanismo, nuestros resultados contribuyen al intenso debate que existe alrededor de los mecanismos que subyacen a la muerte de los árboles en condiciones de sequía (McDowell & Sevanto 2010; Sala *et al.* 2010; McDowell *et al.* 2011), aportando medidas directas de las reservas de carbono almacenadas en el tronco y demostrando que una especie isohídrica como el pino albar puede llegar a agotar sus reservas.

En nuestro caso, además del cierre estomático, la reducción del área fotosintética por la caída de las hojas durante la sequía parece ser un mecanismo sumamente importante que limita la asimilación de carbono y acaba resultando en el agotamiento de las reservas a largo plazo. Otros trabajos recientes han medido las reservas de carbono en condiciones de estrés hídrico en distintos órganos (hojas, ramas, troncos y raíces) de pino albar y de una especie de álamo americano sin encontrar ningún indicio de agotamiento (Gruber *et al.* 2011; Anderegg *et al.* 2012), aunque los niveles de estrés hídrico considerados en Gruber *et al.* no son tan extremos como los estudiados en esta tesis. McDowell *et al.* (2011) han planteado recientemente la estrecha interdependencia entre el transporte hidráulico y la disponibilidad de carbono, así como la importancia de tener en cuenta la intensidad y la duración de la sequía. Además, otros estudios han propuesto otros mecanismos como son la inmovilización de reservas o la fallida del transporte floemático que podrían llevar a la muerte de los árboles por inanición de carbono sin que necesariamente hubieran agotado sus reservas (Sala *et al.* 2010). Todo esto, junto con las particularidades que las especies y/o fases ontogénicas presentan en relación a la regulación del cierre estomático y la economía del carbono, explica el amplio rango de respuestas encontradas en la bibliografía. Se necesitan muchos más estudios comparativos, tanto experimentales como en condiciones naturales, que ayuden a establecer patrones generales en la vulnerabilidad de las distintas especies, taxones, ecosistemas o biomas a los eventos climáticos extremos, y así avanzar en los modelos de predicción de las respuestas de la vegetación a las proyecciones del cambio climático (ver McDowell *et al.* 2011).

Cambios en los patrones de reclutamiento

Las sequías extremas pueden producir extensas mortalidades en ecosistemas forestales (Allen & Breshears 1998; Allen *et al.* 2010). Sin embargo, la capacidad de estos eventos de inducir cambios en la vegetación viene determinada por el balance entre la mortalidad y el reclutamiento de las distintas especies de la comunidad (Lloret *et al.* 2012). Los resultados de esta tesis muestran que las pérdidas en el dosel del pino albar inducidas por la sequía del 2004-2005 no se ven compensadas por el reclutamiento de nuevas plántulas de esta especie, lo cual indica que dichas aperturas en el dosel no proporcionaron las condiciones adecuadas para la regeneración (cf. Castro *et al.* 2004). Por el contrario, y a pesar de la escasa presencia de adultos, otras especies de árbol (*Quercus humilis* Mill. y *Q. ilex* L.) que potencialmente podrían reemplazar al pino albar mostraron un alto reclutamiento en las partes intermedias y bajas de la ladera estudiada, coincidiendo con las zonas donde el pino se vio más afectado por la sequía. No obstante, los patrones espaciales de regeneración de las especies de *Quercus* en relación a la mortalidad del pino albar fueron complejos, con importantes diferencias entre el crecimiento de plántulas preexistentes y el establecimiento de nuevas. Las aperturas del dosel creadas por los efectos de la sequía en las copas de pino albar probablemente causaron una reducción de la limitación por luz en el sotobosque, lo cual resultó en una respuesta inmediata de aumento de crecimiento en el banco de plántulas. Aunque existen trabajos que documentan respuestas de crecimiento en procesos de apertura del dosel en bosques (Van Der Meer *et al.* 1999; Wilder *et al.* 1999), los estudios específicos en el ámbito de la mortalidad en bosques inducida por sequía son muy escasos (Suarez & Kitzberger 2008). Elevadas tasas de crecimiento proporcionan a las plántulas ventajas competitivas de supervivencia y reproducción que pueden facilitar la expansión de estas especies a través del establecimiento de nuevos propágulos (cf. Walters & Reich 2000).

La abundancia de plántulas de *Quercus* fue también mayor bajo doseles defoliados de pino albar, aunque esto no se debió a la emergencia de nuevas plántulas de *Quercus* después del episodio de sequía del 2004-2005, la cual fue incluso inferior bajo doseles defoliados. Estos resultados sugieren que las condiciones más secas en las aperturas del dosel originadas por el episodio de sequía limitaron la capacidad de las plántulas de *Quercus* para afrontar el estrés hídrico en las etapas tempranas del establecimiento (Espelta 1996; Rey Benayas 1998; Retana *et al.* 1999). Por lo tanto, las elevadas densidades de plántulas de *Quercus* en zonas defoliadas estarían más relacionadas con la elevada densidad inicial de plántulas en aquellos sitios que finalmente acabarían siendo más afectados por la sequía del 2004-2005. Esta diferencia inicial en el número de plántulas está probablemente relacionada con la propia estructura del rodal. En parcelas con elevados valores de área basal total, la abundancia de plántulas era mayor, lo que sugiere que las condiciones de sombra proporcionadas por los árboles antes del episodio de sequía habría favorecido el establecimiento de plántulas de estas especies que se comportarían como tolerantes a la sombra. Al mismo tiempo, las zonas con mayor competencia entre árboles fueron las más afectadas por la sequía (cf. Galiano *et al.* 2010), y las nuevas condiciones bajo las aperturas del dosel no serían apropiadas para el nuevo establecimiento.

En resumen, los resultados ilustran que la existencia de mecanismos distintos a la emergencia de plántulas de las especies no dominantes, como es el crecimiento de los individuos del banco de plántulas del sotobosque, podría acelerar la dinámica forestal en condiciones de cambio climático. Además del papel de los cambios recientes en el clima, el cese de las actividades silvícolas en los años 80 también ha influido significativamente en la aparente expansión de quercíneas hacia altitudes superiores en la ladera. De todos modos, es fundamental realizar estudios de monitoreo que esclarezcan si los patrones que observamos en este estudio se mantendrán a largo plazo, pudiendo conducir finalmente a cambios en la vegetación.

Conclusiones

I. Los episodios de mortalidad en bosques asociados a eventos climáticos extremos han emergido en los últimos años como un fenómeno que ocurre a escala global. Los efectos sobre los parámetros demográficos, sin embargo, muestran patrones complejos y apuntan a que un amplio rango de factores intervienen en el proceso de declive del bosque. En nuestro caso, la estructura del bosque, las propiedades del suelo, la topografía y la infección por parásitos actúan como factores de predisposición, haciendo que los árboles sean más susceptibles a las sequías climáticas.

II. Muchos árboles aun se están recuperando de los efectos de la sequía varios años después del episodio. Los niveles de reservas de carbono almacenados en el tronco o el lignotuber se relacionan con el estado de recuperación que muestran los árboles, en particular con la cantidad de hojas presentes en el dosel. Los árboles con un peor estado de recuperación mostraban una reducción importante de reservas, lo que supone una pérdida de resiliencia de cara a afrontar nuevos episodios de sequía.

III. Las reservas de carbono tienen un papel clave en la supervivencia y la recuperación de los individuos después de sufrir los efectos de una sequía extrema. La relación entre la cantidad de hojas y de reservas de carbono constituye un potencial mecanismo de retroalimentación que limita la recuperación de los árboles (menos hojas implica menos reservas de carbono y viceversa), dando lugar a recuperaciones prolongadas de los individuos supervivientes, y llegando incluso a producir la muerte de los individuos con menos reservas varios años después del evento de sequía.

IV. En nuestro caso, los patrones de regeneración posteriores a un episodio de mortalidad inducida por sequía proporcionan indicios de posibles cambios futuros en la vegetación. Otras especies de árboles (*Quercus* spp.) mostraban una abundancia de plántulas muy superior a la de la especie dominante en el dosel (pino albar), y en particular en las zonas donde el pino estuvo más afectado. No obstante, los patrones espaciales de regeneración de *Quercus* spp. en relación a la mortalidad del pino albar fueron complejos, con efectos opuestos sobre distintos estadios de regeneración. Así, la mortalidad del pino se asoció positivamente con el crecimiento de las plántulas preexistentes pero negativamente con el establecimiento de nuevas plántulas.

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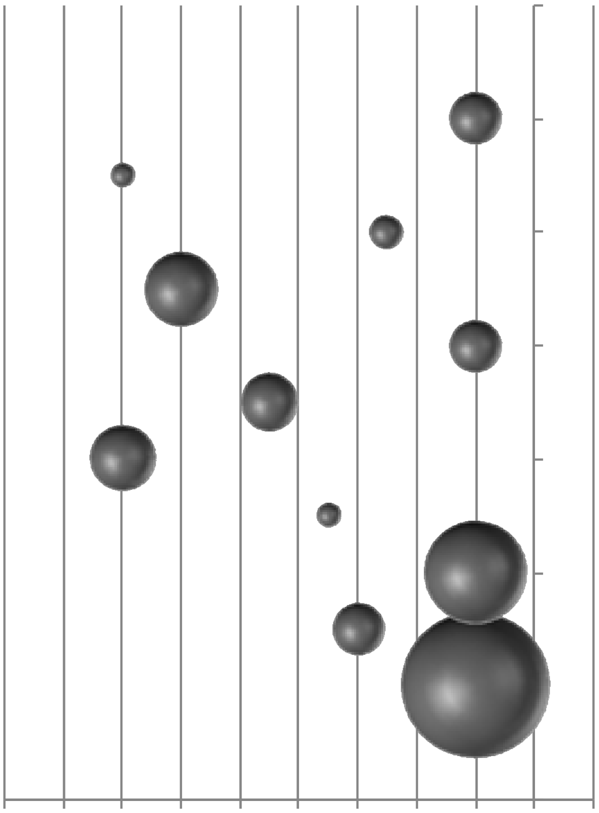
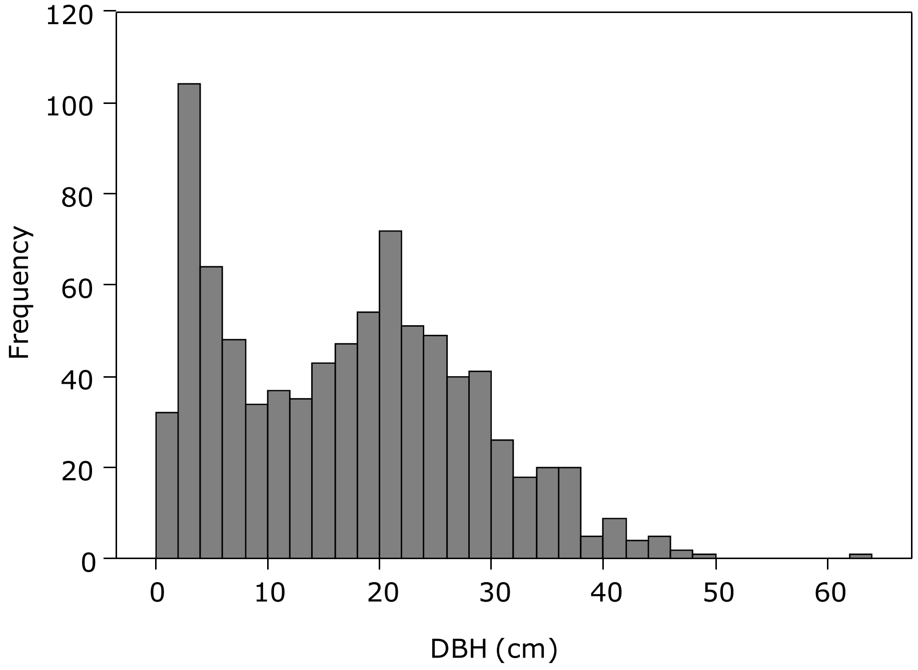
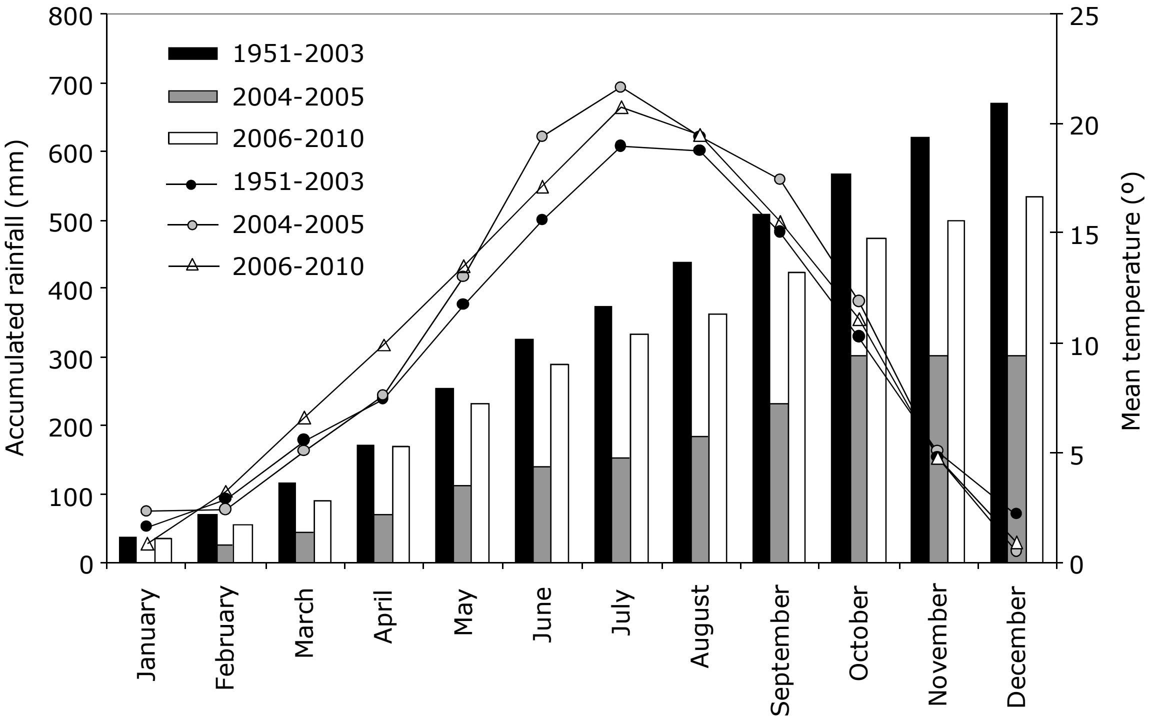


Apéndices

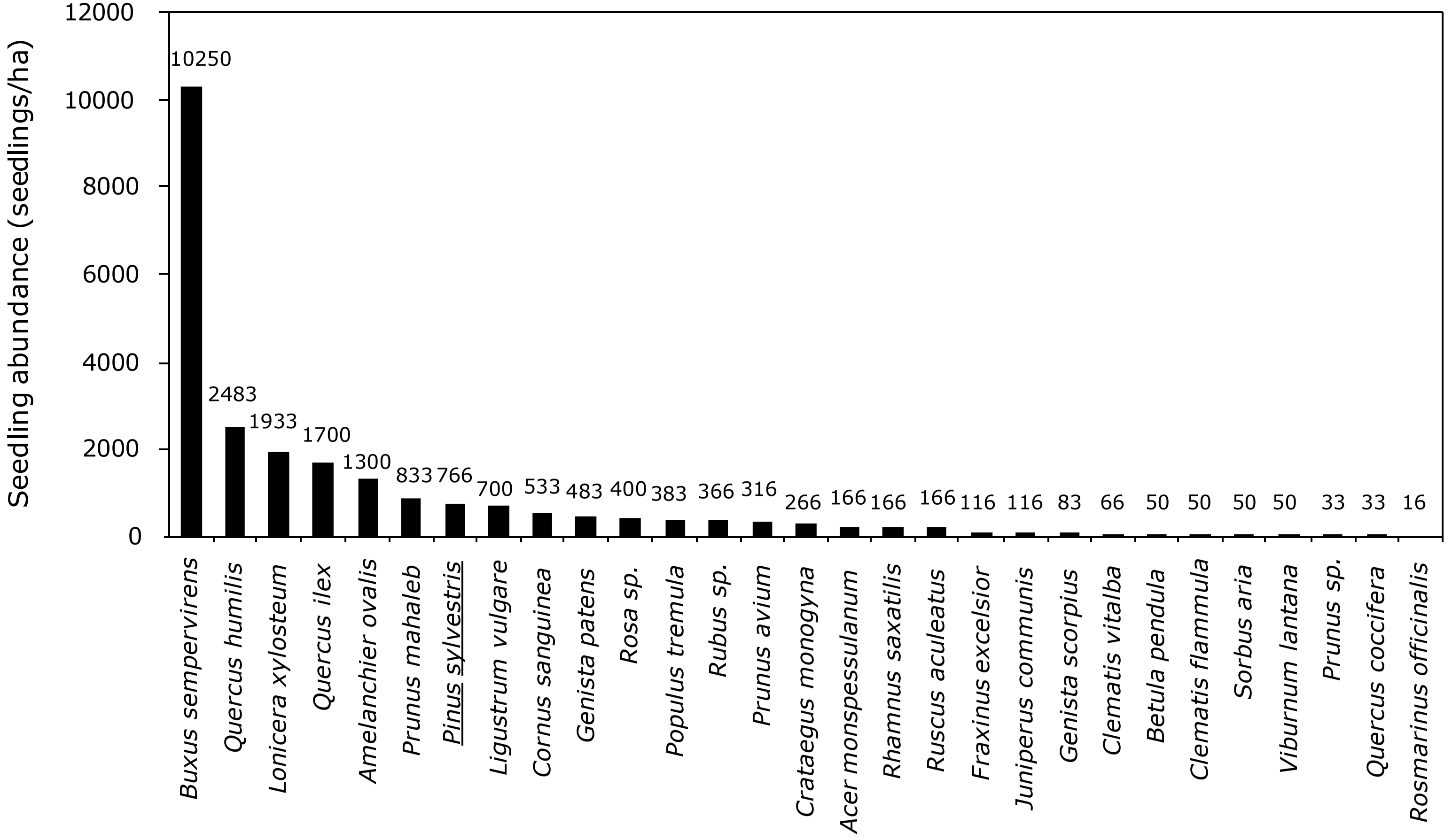
Appendix 2.1. Size (DBH) class distribution of the studied *Pinus sylvestris* L. population. N = 1002.



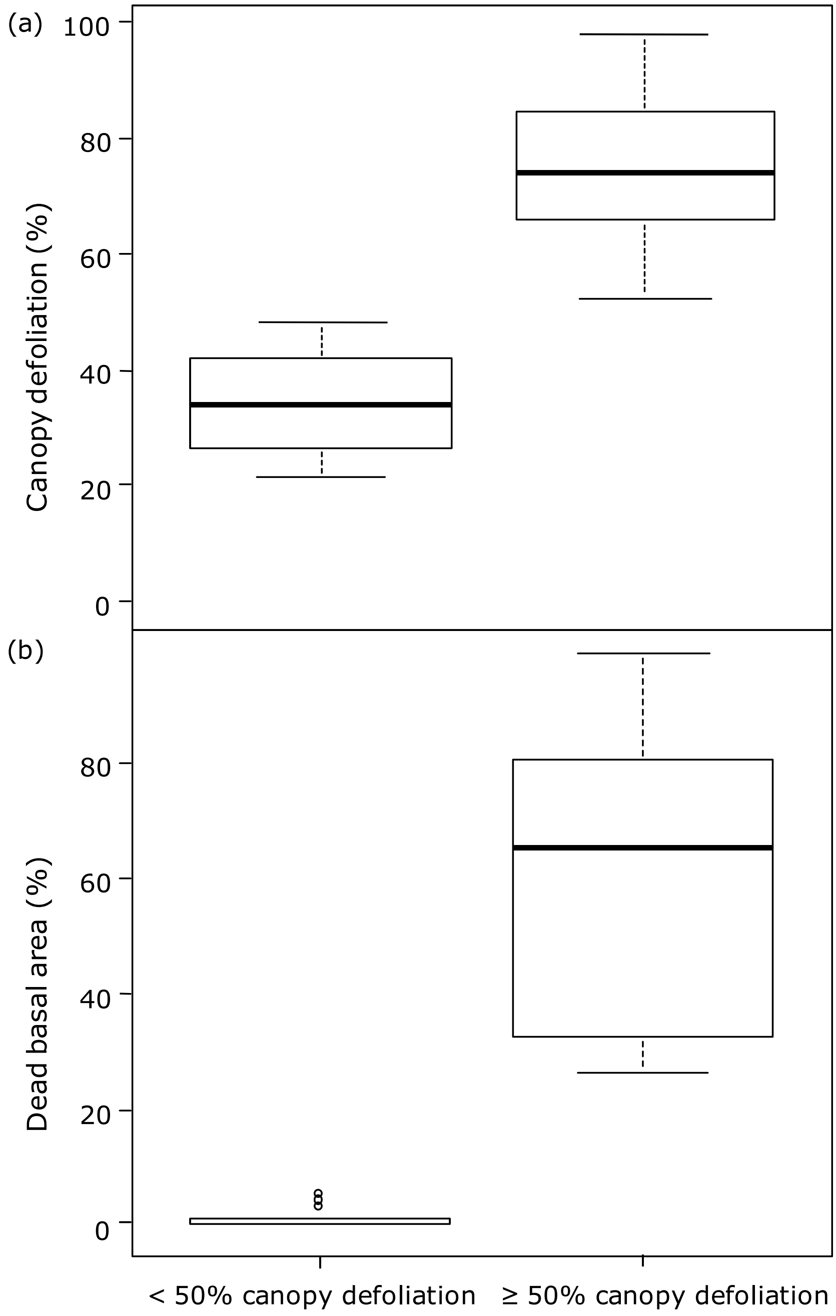
Appendix 2.2. Monthly meteorological data for the studied area (data obtained from the Spanish meteorological station network (Agencia Estatal de Meteorología)). Accumulated rainfall (bars) and mean temperature (lines), from January to December, are shown for the average conditions of the following periods: before (1951-1999), during (2004-2005) and after (2006-2010) the drought episode.



Appendix 2.3. Average seedling abundance of all species found in the study at the plot level.



Appendix 3.1. Box plots of (a) canopy defoliation (%) and (b) dead basal area (%) of *Pinus sylvestris* at the plot level as a function of canopy defoliation class (< 50%, N = 14 and ≥ 50%, N = 13). Boxes indicate the lower and upper quartile, the band inside the box is the median, and whiskers extend to the most extreme data points which are within 1.5 times the interquartile range.



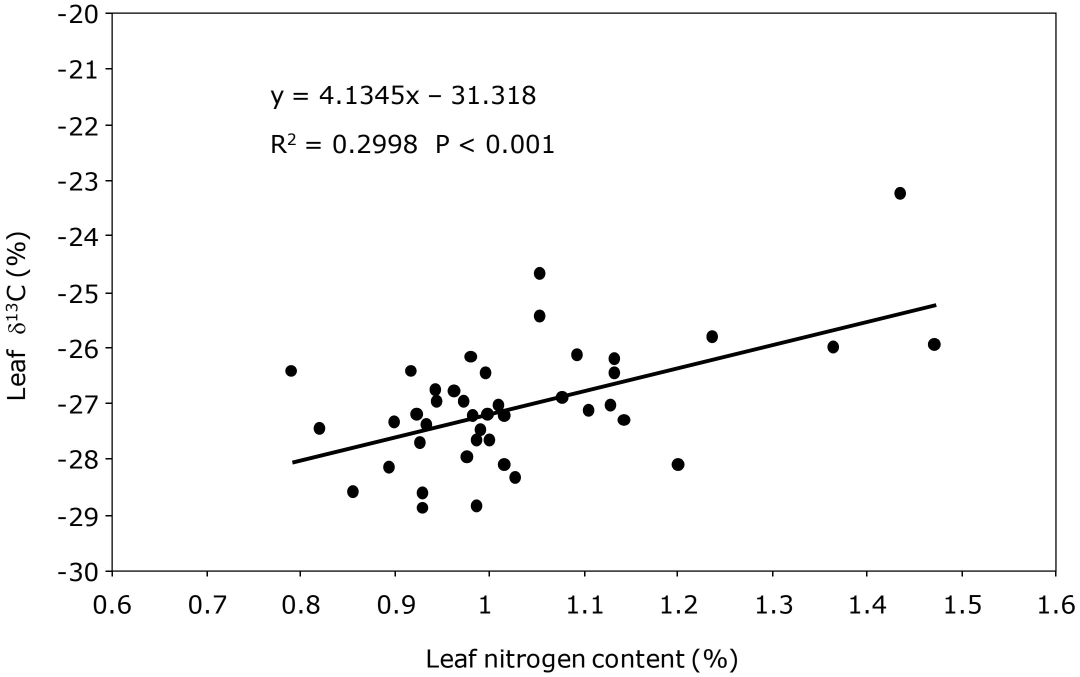
Appendix 4.1. Direct and indirect effects of variables included in the structural equation models for each hypothesis ((a) for hypothesis H1, (b) for hypothesis H2, and (c) for hypothesis H3). Significant direct effects are marked with asterisks (*P < 0.05, **P < 0.01, ***P < 0.001). DE = direct effects, IE = indirect effects.

(a)	Dependent variables									
	Leaf nitrogen content		Leaf δ13C content		Green leaves		Stem sapwood NSC concentrations		Current $G_{2008\text{-}2009}$	
Independent variables	DE	IE	DE	IE	DE	IE	DE	IE	DE	IE
$G_{pre\text{-}drought}/G_{drought}$	0	0	0	0	-0.449**	0	0	-0.222	-0.286*	-0.257
Mistletoe index	-0.366*	0	0.138	-0.224	0	0.020	0.149	0.010	0.190	-0.149
Leaf nitrogen content	0	0	0.611***	0	0	-0.143	0.085	-0.297	0.403***	-0.069
Leaf δ13C content	0	0	0	0	-0.233	0	-0.371*	-0.115	0	-0.101
Green leaves	0	0	0	0	0	0	0.495***	0	0.616***	-0.043
Stem sapwood NSC concentrations	0	0	0	0	0	0	0	0	-0.088	0

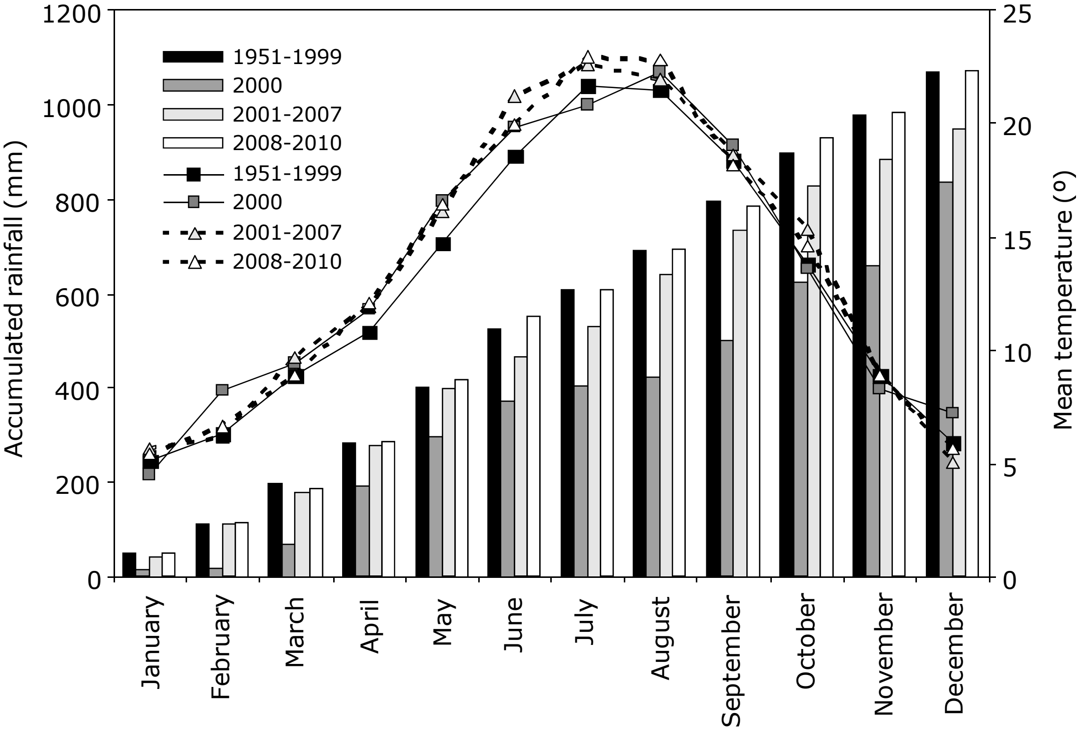
(b)	Dependent variables									
	Leaf nitrogen content		Leaf δ13C content		Green leaves		Stem sapwood NSC concentrations		Current $G_{2008\text{-}2009}$	
Independent variables	DE	IE	DE	IE	DE	IE	DE	IE	DE	IE
$G_{pre\text{-}drought}/G_{drought}$	0	0	0	0	0	-0.235	-0.368*	0	-0.317*	-0.107
Mistletoe index	-0.372*	0	0.137	-0.227	0	0.058	0.063	0.033	0.185	-0.131
Leaf nitrogen content	0	0	0.611***	0	0	-0.137	0.022	-0.278	0.420***	-0.058
Leaf δ13C content	0	0	0	0	0.043	-0.291	-0.454**	0	0	-0.106
Green leaves	0	0	0	0	0	0	0	0	0.628***	0
Stem sapwood NSC concentrations	0	0	0	0	0.640***	0	0	0	-0.110	0.402

(c)	Dependent variables									
	Leaf nitrogen content		Leaf δ13C content		Green leaves		Stem sapwood NSC concentrations		Current $G_{2008\text{-}2009}$	
Independent variables	DE	IE	DE	IE	DE	IE	DE	IE	DE	IE
$G_{pre\text{-}drought}/G_{drought}$	0	0	0	0	-0.445**	0	-0.329*	0	-0.311**	-0.241
Mistletoe index	-0.365*	0	0.133	-0.223	0	0.021	0.109	0.011	0.179	-0.144
Leaf nitrogen content	0	0	0.610***	0	0	-0.144	0.089	-0.298	0.400***	-0.068
Leaf δ13C content	0	0	0	0	-0.235	0	-0.488**	0	0	-0.099
Green leaves	0	0	0	0	0	0	0	0	0.608***	0
Stem sapwood NSC concentrations	0	0	0	0	0	0	0	0	-0.091	0

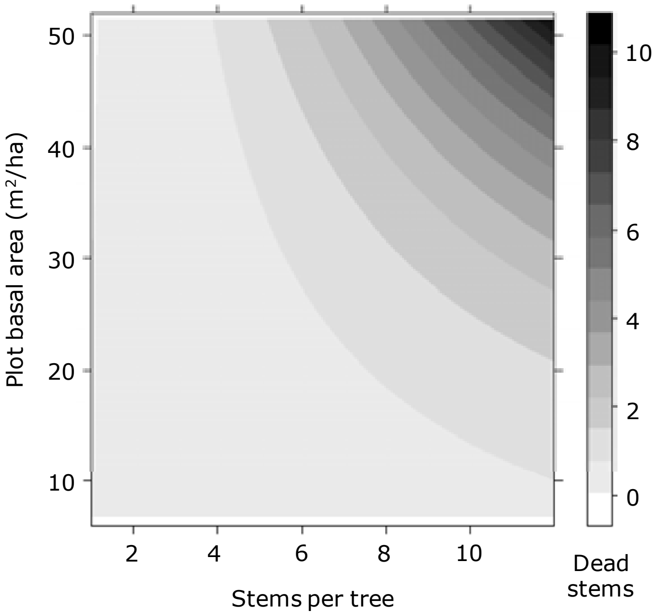
Appendix 4.2. Relationship between leaf $\delta^{13}C$ and leaf nitrogen content of current needles (2009). Regression equation, R^2 and P value are shown. N = 42 individuals.

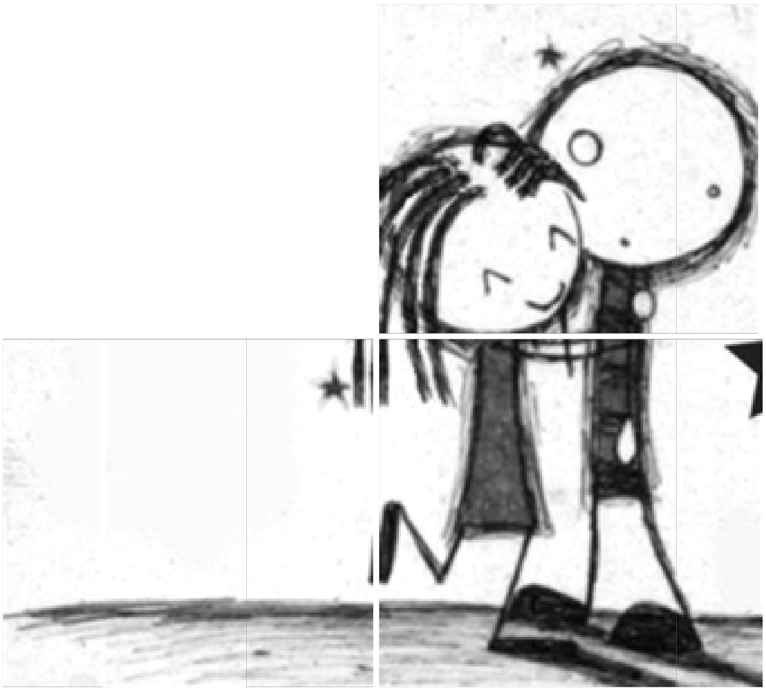


Appendix 5.1. Monthly meteorological data for the studied area (data obtained from the Spanish meteorological station network (Agencia Estatal de Meteorología)). Accumulated rainfall (bars) and mean temperature (lines), from January to December, are shown for the average conditions of the following periods: prior to the drought episode (1951-1999), during the drought episode (2000), since the date of the drought episode until the first sampling (2001-2007) and between the first and the second samplings (2008-2010).



Appendix 5.2. Surface plot showing the predicted effect (see Table 2b) of the interaction between stems per tree and transect basal area on the number of dead stems per tree. Observations (N= 368) cover the whole space represented in the plot.





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Mis niñas de Lliçà (Irene, Miri y Tamy). Inseparables desde los 8 años, no ha habido tregua. Todavía nos recuerdo jugando en el patio del cole, que rápido ha pasado el tiempo. Sois parte de mí, como hermanitas, gracias por saber lo que necesito antes siquiera pueda abrir la boca. Aix…nos conocemos tanto…confianza absoluta. Gracias. Por no importarnos a ninguna rozar los 30 y sacar siempre la máxima expresión del niño que llevamos dentro…Porque cuando estamos juntas se crea una burbuja a nuestro alrededor, jaja... Miri ahora dirás que a ver en que me lio ahora, jaja… Pero así somos, cada una en nuestros mundos particulares de *yupi*, pero con una conexión total.

A toda mi familia. Por ser un ejemplo claro de valentía y superación. Por ser la mejor. Una familia humilde, que siempre ha sabido transmitir unos buenos valores, sin ningún resquicio, a las nuevas generaciones. Por vosotros existo y por vosotros soy. A todos mis primos y primas, tíos y tías, a mis abuelos por ser una familia tan unida, y por enseñarme a demostrar lo que siento sin complejos. Gracias. A mis padres, por haber luchado contra viento y marea para poder darme lo que siempre he necesitado, todo en el momento justo y de manera justa. Gracias por transmitirme el amor tan profundo que sentís por la naturaleza, el respeto y la curiosidad por todo lo que hay en ella. Gracias a mi hermana, porque como muy buena hermana mayor siempre me ha mostrado absoluta predilección y protección. Gracias por todo vuestro apoyo y paciencia. Ya sabéis cuantísimo os quiero. Siempre, y a por todo! La vida es una constante evolución.

Al meu príncep, el noi més dolcet que existeix a la faç de la Terra. Per fer-me sentir sempre tan especial. Per ser ell tan especial i estar sempre al meu costat (soy una suertuda ;-P). I perquè com diuen els Manel: ell és més de sofá i Barça, jo sóc més de sortir...ens ha costat Dèu i ajuda arribar fins aquí! jeje...Perquè t'estimo moltíssim, perquè ets la meva altra meitat que em complementa i m'estabilitza. Gràcies per donar-me pau i tranquil·litat. I bé, no deixaré de dir lo molt que també m'has ajudat en aquesta etapa, tant professional com mentalment. No ens hem conegut de cap altra manera, i espero impacient les noves cosetes que ens esperen! Ja sabem que per nosaltres els kilòmetres que de vegades ens separen només és distància. Sempre connectats. Gràcies per tot!

Al final me he pasado un poco de las páginas que había prometido…;-P ¿Será por qué soy muy afortunada?