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TESIS DOCTORAL

RASGOS FUNCIONALES Y SU RELACIÓN CON EL FUNCIONAMIENTO DE LAS PLANTAS

Autora: Bárbara López-Iglesias

Director: Rafael Villar Montero

Córdoba, diciembre de 2014

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Este libro se ha impreso utilizando papel procedente de una gestión forestal sostenible y fuentes controladas, todo ello aplicando buenas prácticas para la sosteniblidad editorial, dentro del Proyecto Life+ Ecoedición de la Consejería de Medio Ambiente y Ordenación del Territorio de la Junta de Andalucía.

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TÍTULO DE LA TESIS
Rasgos funcionales y su relación con el funcionamiento de las plantas

DOCTORANDO
Bárbara López Iglesias

INFORME RAZONADO DEL DIRECTOR DE LA TESIS

El trabajo presentado por Bárbara López Iglesias titulado "Rasgos funcionales y su relación con el funcionamiento de las plantas" constituye la memoria de su tesis doctoral. El tema central de su tesis, los rasgos funcionales de las plantas, se definen como los atributos físicos y químicos de las plantas que pueden ser utilizados como indicadores o predictores del funcionamiento de las plantas y de sus respuestas ante distintos factores ambientales. En esta tesis se concluye cómo estos rasgos funcionales están muy relacionados con el crecimiento de las plantas, con la resistencia a la sequía, las características de la hojarasca, la descomposición y la liberación de nutrientes. Por todo ello, los rasgos funcionales influyen de manera decisiva en el funcionamiento de los ecosistemas terrestres.

Esta tesis consta de 7 capítulos: una introducción general, 5 capítulos experimentales y una discusión general. La temática es variada, incluyendo el crecimiento de plántulas y árboles adultos (capítulo 2), los efectos de la sequía (capítulos 3 y 4), la influencia de los rasgos funcionales de la hoja verde sobre la hojarasca (capítulo 5) y la descomposición de la hojarasca (capítulo 6).

El presente trabajo supone una aportación importante al campo de la ecología funcional de especies mediterráneas como lo demuestra el hecho de que varios de los capítulos han sido publicados en revistas internacionales del primer y tercer cuartil según el Journal Citation Report (JCR). Además, Bárbara López Iglesias ha presentado varias comunicaciones a congresos nacionales e internacionales y ha colaborado durante su doctorado en otros trabajos que están en vías de publicación y que avalan su completa formación.

Bárbara López Iglesias cumple con los requisitos académicos y científicos exigidos para la defensa de la tesis y para poder optar al grado de Doctor en Biología.

Por todo ello, se autoriza la presentación de la tesis doctoral.

Córdoba, 15 de diciembre de 2014

Fdo: Rafael Villar Montero

INFORME SOBRE APORTACIONES DERIVADAS DE LA TESIS DOCTORAL Y FACTOR DE IMPACTO DE LAS REVISTAS CIENTÍFICAS (JOURNAL CITATION REPORTS)

Publicaciones en revistas científicas (Capítulos de la Tesis)

Villar R., **Lopez-Iglesias B.**, Ruiz-Benito P., Zavala M. A. y De la Riva, E. G. (2014). Crecimiento de plántulas y árboles de seis especies de *Quercus*. *Revista Ecosistemas 23*: 64-72. **(CAPÍTULO 2 DE LA TESIS)**

Factor de impacto: en proceso de evaluación por parte de Thomsons Routers para entrar en el Scientific Citation Index (SCI).

Lopez-Iglesias B., Poorter L. and Villar R. (2014) Functional traits predict drought performance and distribution of Mediterranean woody species. *Acta Oecologica 56*: 10-18. **(CAPÍTULO 3 DE LA TESIS)**

Factor de impacto: 1.84. Posición de la revista en relación a su categoría específica (Ecology): 74/121; tercer cuartil (Q3).

Olmo M., **Lopez-Iglesias B.**, and Villar R. (2014). Drought changes the structure and elemental composition of very fine roots in seedlings of ten woody tree species. Implications for a drier climate. *Plant and Soil 384*: 113-129. **(CAPÍTULO 4 DE LA TESIS)**

Factor de impacto: 3.23. Posición de la revista en relación a su categoría específica (Plant Sciences): 32/199; primer cuartil (Q1).

Lopez-Iglesias B., Olmo M., Gallardo A., and Villar R. (2014). Short-term effects of litter from 21 woody species on plant growth and root development. *Plant and Soil*, 1-15. **(CAPÍTULO 6 DE LA TESIS).**

Factor de impacto: 3.23. Posición de la revista en relación a su categoría específica (Plant Sciences): 32/199; primer cuartil (Q1).

Otros trabajos en los que ha colaborado

Salazar P., Villar R., **Lopez-Iglesias** B. and Ubera J.L. Leaf structure and functioning in ten Mediterranean woody species. Manuscrito enviado a *Tree Physiology.*

Bongers FJ, Olmo M., **Lopez-Iglesias B**., Anten NPR, Villar R. Phenotypic plasticity and drought performance of Mediterranean species in two different environmental conditions. Manuscrito enviado a *Functional Ecology*.

Comunicaciones presentadas en congresos nacionales

Lopez-Iglesias B., Villar R., y Poorter L. (2013). Rasgos funcionales como indicadores de la respuesta a la sequía en plántulas de 10 especies leñosas mediterráneas. Actas 6 º Congreso Forestal Español. Póster.

Lopez-Iglesias B. and Villar R. (2012). Rasgos vegetales: causas y consecuencias sobre el funcionamiento de plantas y ecosistemas mediterráneos. Congreso Científico de Investigadores en Formación en Agroalimentación (CeiA3) y II Congreso Científico de Investigadores en Formación de la Universidad de Córdoba. Póster.

Lopez-Iglesias B., Olmo M., Gallardo A., y Villar R. (2013) Efectos de la hojarasca de 21 especies leñosas sobre el crecimiento de las plantas. II Congreso Científico de Investigadores en Formación en Agroalimentación (CeiA3) y III Congreso Científico de Investigadores en Formación de la Universidad de Córdoba. Comunicación oral.

Otras comunicaciones

Villar, R., **Lopez-Iglesias B**., Ruíz-Benito P., Quero J.L. (2013). Growth of *Quercus* and responses to environmental factors. Environment Workshops 2013: 'Oak forests coping with global change: Ecology and management'. Universidad Internacional de Andalucía (UNIA). Comunicación oral realizada por R Villar.

‘No sabía muy bien a qué iba a dedicarse. No paraba de darle vueltas. Un día recibió una llamada: había sido seleccionada para buscar una lección escondida. Comenzó con curiosidad, pero al tiempo empezó a dudar de sí misma. Aquella tarea que le habían encomendado era casi imposible.

Cada día iba al bosque y miraba hacia arriba, pero su lección no estaba allí. No era capaz de encontrarla. Pedía ayuda a su maestro, él conocía bien la manera. Le decía: -la lección la tienes ahí, sólo tienes que buscarla con más esfuerzo-.

Pasaron muchos días así, hasta que empezó a dudar de su maestro. Pensaba que la lección no existía o que quizá él ya no quería entregársela. Parecía que su esfuerzo nunca era suficiente.

Pedía ayuda al bosque: cerraba los ojos y escuchaba los mensajes de los árboles. Pero no los entendía y volvía y marcharse: -no soy capaz ni de entender a los árboles-. Buscaba en los escritos, pero no comprendía bien su lenguaje: -no soy capaz de entender los escritos-. Estaba perdiendo la esperanza de encontrar su lección…-¿Por qué he sido elegida? ¿Por qué acepté buscarla? No seré capaz de dar con ella-.

Tendría que aceptar que ese trabajo no estaba hecho para ella. Necesitaba visitar otros bosques, leer otros escritos, hablar con otras gentes. Se sentía como en una trampa sin salida.

Los animales del bosque, que tantos días la habían visto por allí, comenzaron a echarla de menos. Ellos, que habían permanecido en ese bosque por largo tiempo, sabían dónde estaba la lección y no entendían por qué ella no podía verla. Se organizaron con el maestro para darle todo su apoyo en algo que para ellos estaba tan claro. La convencieron de que la lección estaba allí para todos, y que también lo conseguiría. Se ofrecieron a permanecer junto a ella en el bosque durante este último intento. Accedió, sintiendo que esta vez era diferente. Pasaron varios días, varias noches. Pero la lección no aparecía para ella. Empezó a sentirse mal de nuevo, no podía, ya estaba claro. Estaba dispuesta a marcharse y dejarlo. Pero esta vez no estaba sola. Sus amigos le aconsejaron:

-Lucha. Eres igual que nosotros. La verás, al menos esta vez. Quédate un rato más-.

Suspiró hondo y cerró los ojos. Parecía que no iba a acabar nunca. Oía voces en su interior:-vámonos, no puedes, déjalo, aunque decepciones a todos-. Empezó a secarse las lágrimas, dispuesta a marcharse. Pero, de repente, el viento empezó a soplar. Los árboles comenzaban aquella hechizante cháchara, que aunque familiar, le resultaba siempre ininteligible. Pero esta vez era distinto: empezó a recordar veces anteriores, aquellas largas horas que pasaba en el bosque, y a darse cuenta de que todo tenía una lógica. Poco a poco fue encajando todas las piezzas del puzzle y a descifrar aquél código misterioso: raíces, madera, hojas, mira…mira…mira…

Abrió los ojos y observó los árboles. Toda su estructura: raíces, tronco, ramas, hojas…Los había visto cientos de veces, pero nunca los había contemplado como aquél día. Se detuvo en las caprichosas formas de sus ramas. Hoy le parecían unas rudimentarias runas. Se fijó mejor y advirtió que una tenía forma de N. Se frotó los ojos y vió una A. Se restregó la cara y, allí, donde había estado miles de veces, triste, desesperada, atrapada, estaba escrita, nítida, Su Lección.

Todos se dieron cuenta de que lo había conseguido y se cogieron de las manos para celebrarlo y darle un último empujón. El rumor de los árboles y sus voces se fundieron en una sola para entonar esos milenarios cánticos:

Nada se consigue sin esfuerzo.'

Índice

Capítulo 1. Introducción general

Los tres factores principales que limitan y afectan a las plantas durante su ciclo de vida son la luz, el agua y los nutrientes (Lambers et al. 2008). Así, a lo largo de su existencia, las plantas tienen que enfrentarse a distintos "retos" que deben resolver y superar para sobrevivir y reproducirse. Estos retos serán, entre otros, el exceso de luz o la sombra, la inundación o la sequía, el exceso o la escasez de nutrientes, el calor o las heladas, la competencia intra e inter-específica, las plagas y la herbivoría (Lambers et al. 2008).

De este modo, en determinadas ocasiones extremas, las plantas tendrán que "elegir" entre crecer o sobrevivir (Fig. 1 ; Sterck et al., 2006), manteniendo siempre un balance de carbono positivo, unos órganos funcionales, y un reciclaje de nutrientes eficiente.

Para ello, utilizarán un amplio abanico de distintas respuestas y estrategias, relacionadas con las características específicas con las que cuenta cada especie. Estas características o propiedades son mi objeto de estudio, definiendo a los principales protagonistas de esta tesis: **los rasgos vegetales**. Éstos, también llamados *rasgos funcionales*, se definen como atributos físicos y químicos de las plantas que sirven como indicadores o predictores de las respuestas de las plantas ante factores ambientales (Lavorel y Garnier 2002; Cornelissen et al. 2003).

Por ejemplo, se ha encontrado que los rasgos funcionales están muy relacionados con el crecimiento de las plantas (Fig. 1): el área específica foliar (*specific leaf area,* SLA), la asimilación neta (*net assimilation rate,* NAR), o la razón de área foliar (*leaf area ratio,* LAR), son buenos predictores de las tasas máximas de crecimiento relativo (*relative growth rate,* RGR; Poorter y Remkes 1990; Garnier 1992; Lambers y Poorter 1992; Antúnez et al. 2001).

En relación a la sequía, se ha observado que las plántulas con hojas de área pequeña (*leaf area*, LA) y una alta relación entre la biomasa de raíces y parte aérea (*root:shoot*, R:S) son capaces de soportar mejor la sequía que plántulas que posean los rasgos opuestos (Fig. 1; Leiva y Fernández-Aléz 1998). Atendiendo al tallo, una alta densidad de la madera permite a las plantas tener una mayor resistencia a la sequía en plántulas en bosques tropicales (Fig. 1; Markesteijn et al. 2011). Además, se ha comprobado que las especies leñosas presentes en ambientes secos con largas estaciones de sequía, tienen raíces más profundas (Filella y Peñuelas 2003; Sack et al. 2003; Markesteijn y Poorter 2009), y un mayor cociente entre la profundidad máxima de raíz y el área foliar (Fig. 1; Paz 2003).

Todos los rasgos mencionados hasta ahora son estructurales, pero además de la estructura, la composición química de la hoja (sin considerar al carbono, que forma compuestos con función estructural, entre otras), también tiene un papel muy importante en el funcionamiento de las plantas. Por ejemplo, la concentración de nitrógeno foliar (N) se ha descrito como un rasgo que explica la RGR (Huante et al. 1995; Cornelissen et al. 1996; Villar et al. 2006; Villar et al. 2008). Además, la concentración de nutrientes se considera indicadora del estatus nutricional de la planta, y ha sido relacionada con la eficiencia en la reabsorción de nutrientes (Kobe, Lepczyk y Iyer 2005). La composición química de la hojarasca es indicadora de su calidad: una hojarasca con alta concentración de nutrientes se considera de mayor calidad, y ésta afecta de manera importante a la tasa de descomposición, acelerándola (Gallardo y Merino 1993), y facilitando así el regreso de los nutrientes de la hojarasca el suelo, entrando de nuevo en el ciclo de nutrientes. Es más, la calidad de la hojarasca (determinada por su composición química), puede determinar los efectos de la misma sobre el suelo y las plantas, potenciando (Dorrepaal et al. 2007) o inhibiendo (Bonanomi et al.,2011) el crecimiento de las plantas. Por ejemplo, se ha comprobado que rasgos como la concentración inicial de nitrógeno en la hojarasca o la relación C/N pueden usarse como predictores de los posibles efectos de la hojarasca sobre el crecimiento de las plantas (Fig. 1; Doorrepaal et al. 2007).

Si hacemos una visión de conjunto, podemos observar que la asociación de ciertos rasgos funcionales suele ser consistente y similar en distintas comunidades vegetales (Díaz et al. 2004; Wright et al. 2004), y que dichas asociaciones o grupos de rasgos pueden usarse para describir estrategias vegetales. Así, diversos autores han descrito dos estrategias opuestas: especies cuyos rasgos favorecen el uso conservativo de los recursos, y aquellas especies cuyas características les permiten un rápido crecimiento a corto plazo consumiendo rápidamente los recursos (Wright et al. 2001; Poorter et al. 2004; Lopez-Iglesias et al. 2014). Las hojas de las *especies conservativas* suelen tener una elevada longevidad (*leaf life-span*, LL), una alta relación de masa por área foliar (*leaf mass per area*, LMA), baja concentración de nutrientes y baja capacidad fotosintética (Reich, Walters y Ellsworth 1997); las especies en el otro extremo del espectro (*especies adquisitivas*), suelen mostrar características opuestas.

El funcionamiento de las especies y los ecosistemas está estrechamente relacionado con el clima. En las últimas décadas, se han dedicado muchos esfuerzos en demostrar que el cambio climático es un hecho tangible. El calentamiento en el sistema climático es inequívoco y, desde la década de 1950, muchos de los cambios observados no han tenido precedentes en los últimos decenios a milenios. La atmósfera y el océano se han calentado, los volúmenes de nieve y hielo han disminuido, el nivel del mar se ha elevado y las concentraciones de gases de efecto invernadero han aumentado (Stoker et al., 2013). Paralelamente, estos cambios están modificando la composición y funcionamiento de los ecosistemas, reduciendo la producción primaria (McDowell et al. 2008) y disminuyendo los sumideros de carbono, provocando mayores cambios en el clima (Adams et al. 2010). Los impactos de tales cambios en las propiedades de los ecosistemas pueden ser atribuidos a los cambios en las características funcionales de las especies que lo componen. Por tanto, el conocimiento de estas características funcionales de las plantas puede ayudar a predecir los efectos del cambio global sobre los ecosistemas.

A lo largo de esta tesis proponemos un repaso a algunos de los distintos procesos que llevan a cabo las plantas a lo largo de su vida y que tienen papeles muy importantes a nivel de la estructura y funcionamiento de los ecosistemas mediterráneos. Estos procesos, junto con más ejemplos de rasgos funcionales, pueden verse en la Figura 1 y se introducen a continuación:

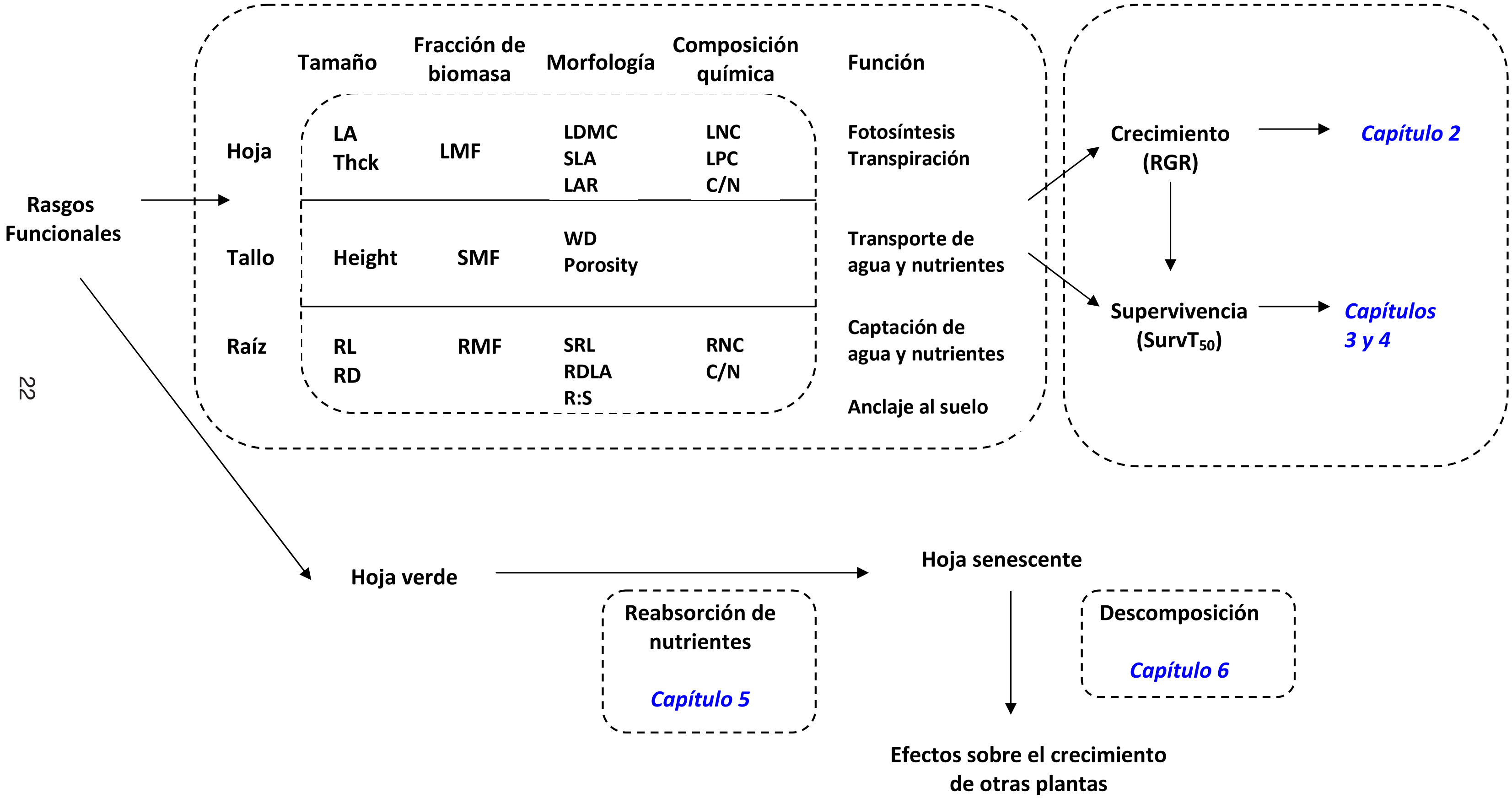

Figura 1. Relación entre los principales rasgos vegetales y los distintos procesos de las plantas estudiados en esta tesis, así como los capítulos en los que se estudia cada uno de ellos. Figura confeccionada a partir de Markesteijn 2010 y Reich 2014.

Crecimiento y su relación con los rasgos funcionales en seis especies de *Quercus*

El crecimiento de las plantas se puede definir como el balance entre la captura y las pérdidas de carbono, nutrientes y agua (Lambers et al., 1998). Suele expresarse como el incremento de biomasa por unidad de tiempo. El crecimiento de las plantas constituye la entrada de energía mayoritaria en los ecosistemas y por tanto de la que dependen los demás niveles tróficos.

Las plantas asimilan la energía luminosa y ésta es almacenada en moléculas de carbono, por lo que las plantas ejercen un papel importante en el ciclo de carbono, actuando a la vez como fuente y sumidero (Dixon et al., 1994). La actividad humana está causando un incremento exponencial de la concentración de CO_2 en la atmósfera (Stoker et al., 2013), y existe una gran preocupación por su papel como efecto invernadero y las consecuencias que puede tener en la modificación del clima. Por ello, es importante conocer las fuentes y sumideros de carbono, para limitar en lo posible el incremento de CO_2 atmosférico.

La Península Ibérica, especialmente en su área mediterránea, se halla dominada por bosques de *Quercus*, tanto perennifolios como caducifolios (Blanco Castro et al., 1997), llegando a constituir hasta un 30 % del área basimétrica total, (Inventario Forestal Nacional, IFN3). Especies de este género, como son *Q. ilex* o *Q. suber* tienen además una gran importancia tanto económica (principalmente ganadera) como etnológica.

El Inventario Forestal Nacional es una herramienta diseñada para describir la estructura, densidad y composición de los bosques de un país. En España se han realizado tres inventarios (el último en 2007). Esta base de datos nos proporciona medidas del diámetro y altura de un mismo pie en un intervalo de diez años. A partir de estas medidas, podemos estimar el crecimiento de un árbol en altura, en diámetro, y, utilizando las ecuaciones alométricas de Montero et al. (2005), en incremento de biomasa. Esta última aproximación es muy conveniente, debido a que gracias a ella podemos también conocer el crecimiento de la fracción subterránea, algo difícil de explorar en condiciones de campo (Poorter et al. 2012).

Existe una gran variación en la tasa de crecimiento relativo entre las distintas especies. Múltiples estudios se han realizado para determinar el crecimiento de plántulas de *Quercus* y cómo éste se ve afectado por los factores ambientales (Quero et al. 2006; Sánchez Gómez et al. 2006). Otros estudios (Antúnez et al. 2001; Ruíz-Robleto y Villar 2005; López-Iglesias et al. 2014) han estimado las tasas máximas de crecimiento relativo, y para ello se han realizado ensayos en condiciones controladas (en invernadero, usando niveles de agua y nutrientes óptimos para el crecimiento).

Hay que reconocer que los valores obtenidos en condiciones controladas y en plántulas podrían diferir de aquellos medidos en condiciones de campo y en adultos. No existen trabajos que comparen diferentes tasas de crecimiento de distintas especies de *Quercus* en condiciones controladas y en campo.

También sería interesante conocer a qué rasgos funcionales se asocian dichas diferencias en el crecimiento entre especies y entre plántulas y adultos de una misma especie.

El objetivo general de este capítulo (**Capítulo 2**) es encontrar y comparar rasgos funcionales asociados al crecimiento de las plantas en dos fases de su vida: fase de plántula y fase de adulto, y en dos entornos distintos de crecimiento: condiciones controladas y en campo. También nos interesa conocer los patrones de inversión de la biomasa en hoja, tallo y raíz en las especies de *Quercus* en condiciones de campo y determinar las tasas de crecimiento en altura, diámetro y biomasa en especies de *Quercus* en adultos y en condiciones de campo.

Para alcanzar nuestros objetivos, para la fase de plántula, utilizamos datos de crecimiento y rasgos funcionales de plántulas en invernadero de siete especies del género Quercus y de 17 especies leñosas (listado de especies en Villar et al., 2008). Estos datos han sido extraídos de trabajos parcialmente publicados. Los rasgos funcionales que utilizamos son el peso fresco de semilla (g), área específica foliar (SLA, m^2 kg^{-1}), concentración de nitrógeno foliar (N), razón de área foliar (LAR, m^2 kg^{-1}), tasa de asimilación neta (NAR, g m^{-2} dia^{-1}), proporción de raíces (RMR, root mass ratio, %) y hojas (LMR, leaf mass ratio, %). Comparamos los valores de los rasgos funcionales con la tasa de crecimiento relativo (RGR, mg g^{-1} $día^{-1}$) en plántulas. Por otra parte, para la fase de adulto nos centramos en seis especies de *Quercus.* Utilizamos datos de rasgos funcionales, como el área específica foliar (SLA) y el contenido en nitrógeno (N) de otros estudios con adultos en campo. A partir de datos del diámetro del tronco y la altura de individuos adultos*,* procedentes de los Inventarios Forestales Nacionales 2 y 3 de España, calculamos la tasa de crecimiento relativo en base a la altura, al diámetro. También en base a la biomasa, empleando las ecuaciones alométricas de Montero et al., (2005). Para conocer los patrones de inversión de biomasa, calculamos valores medios de la proporción de la biomasa en hojas, tallo y raíz (LMR, SMR y RMR, respectivamente) de las especies de *Quercus*, usando los datos de Montero et al. (2005) de biomasa predicha para hojas, tallo y raíz, calculados a partir ecuaciones alométricas en función de las clases diamétricas.

La sequía en el mediterráneo

La disponibilidad de agua es un factor clave limitante para las plantas en muchos ambientes, como los bosques caducifolios templados (Sack 2004), las regiones áridas y semi-áridas (Padilla y Pugnaire 2007) e incluso los bosques tropicales (Engelbrecht et al. 2007). La disponibilidad de agua afecta a la supervivencia y al crecimiento de las plantas (Ciais et al. 2005; Bréda et al. 2006) y, por tanto, a la distribución de las especies (Engelbrecht et al. 2007; Valladares 2008).

Nuestro estudio se centra en los ecosistemas mediterráneos, en los que el establecimiento de las plántulas en los primeros estadíos es especialmente crítico, dado que, durante el verano, una sequía prolongada e intensa coincide con el período más cálido del año (con temperaturas máximas diarias de hasta 45 °C) (Quero et al. 2008; González-Rodríguez et al. 2011). Además, las previsiones para las próximas décadas, indican que la región mediterránea de la Península Ibérica se enfrenta a una disminución del 20% en las precipitaciones y un aumento de 2-3 °C en la temperatura (MARM 2009), lo que limitará aún más la disponibilidad de agua.

Cuando la disponibilidad de agua en el suelo es escasa, el potencial hídrico del suelo baja. La planta necesitará más presión para extraer agua y nutrientes del suelo. Esto lo consigue disminuyendo su potencial hídrico mediante un proceso pasivo resultante del balance hídrico negativo de la planta, creado por una transpiración excesiva (Markesteijn 2010). Una reducción en el potencial hídrico provoca un cierre de los estomas (Zweifel et al. 2007), permitiéndole a la planta reducir una transpiración excesiva y recuperar así su potencial hídrico. De esta manera, evita su desecación y el consiguiente daño a sus tejidos (Bodribb 2009). Pero, un descenso en la conductancia estomática no sólo disminuye la transpiración, sino que además disminuye la asimilación de carbono a través de la reducción del intercambio gaseoso. Si la sequía es prolongada, una caída continuada de la asimilación llevaría a una notable reducción del crecimiento y por tanto, de la productividad de los bosques (Quero et al. 2006; Valladares y Sánchez-Gómez 2006). Además, la presión en los vasos puede ser tan fuerte que éstos se rompan por cavitación, pudiendo resultar finalmente en la muerte de la planta completa (Engelbrecht et al. 2005).

Estos son sólo patrones generales, por lo que es necesario considerar que cada especie ha podido evolucionar de manera distinta para resolver los problemas que la sequía plantea. Esto ha dado lugar a varias estrategias frente a la sequía, que podrían asociarse a distintos grupos de rasgos vegetales. Estos rasgos pueden referirse a la morfología y/o fisiología de la hoja, del tallo o de la raíz.

Esta tesis tiene dos capítulos dedicados a la sequía y los rasgos funcionales.

En el **Capítulo 3**, titulado "*Functional traits predict drought performance and distribution of Mediterranean woody species*", el objetivo principal fue encontrar rasgos funcionales que puedan emplearse para predecir la supervivencia a la sequía en plántulas y la distribución de las especies en un gradiente de humedad.

Para alcanzar estos objetivos llevamos a cabo un experimento de sequía en invernadero en condiciones controladas con plántulas de diez especies leñosas mediterráneas. Se calcularon hasta 17 rasgos funcionales (algunos de ellos en la Fig. 1), como el área específica foliar (SLA), la densidad de la madera (WD), o la longitud específica de raíz (SRL), así como rasgos fisiológicos de las plántulas (como asimilación neta o transpiración). Con estos datos buscamos rasgos vegetales asociados a la supervivencia a la sequía.

Además, a cada especie se le asignó un índice de distribución en relación a la sequía, que denominamos DDI (drought distribution index). Con este se pretende caracterizar las especies en función de su distribución natural y la tolerancia a la sequía (ver Niinemets y Valladares 2006). Utilizamos este índice para comprobar si los rasgos funcionales están relacionados con la distribución de las especies en campo a lo largo de un gradiente de disponibilidad hídrica.

En el **Capítulo 4**, titulado "*Drought changes the structure and elemental composition of very fine roots in seedlings of ten woody tree species. Implications for a drier climate*", el objetivo principal fue conocer rasgos clave de la raíz (concretamente, de las raíces muy finas, con un diámetro menos de 0.5 mm) y comprobar si la sequía puede modificarlos. También queremos saber si la plasticidad de las raíces puede explicar la supervivencia a la sequía.

Para ello, comparamos rasgos funcionales de la raíz entre plantas con riego (control) y plantas en sequía. Estos rasgos son, entre otros: la longitud específica de la raíz (specific root length, SRL), la densidad de tejido de la raíz (tissue mass density, TMDr) y la composición química de la raíz (C y N). Con estas variables podemos saber si la plasticidad de la raíz está relacionada con la supervivencia a la sequía y qué efectos tiene ésta sobre los rasgos de la raíz.

La reabsorción de nutrientes en la hoja

La reabsorción de los nutrientes procedentes de las hojas senescentes es un mecanismo de conservación de nutrientes muy importante, que permite a las plantas usar los mismos nutrientes repetidas veces (Aerts 1996; Wright and Westoby 2003; Yasumura et al. 2005) y ser así menos dependientes de la disponibilidad de nutrientes del suelo cuando éstos escasean (van Heerwaarden et al. 2003). Esto puede ser especialmente ventajoso en ambientes pobres en nutrientes como es el caso del mediterráneo (Gallardo, 2009). Este proceso también es clave porque puede afectar a la calidad de la hojarasca, que determina su tasa de descomposición y por tanto la liberación de nutrientes al suelo. Esto podría explicar los efectos potenciales de las distintas especies en la fertilidad de un suelo (Gallardo y Merino 1993; Cornelissen y Thompson 1997; Perez-Harguindeguy et al. 2000; Norris et al., 2012; Lopez-Iglesias et al., 2014).

La reabsorción de nutrientes ha sido asociada al estatus nutricional de la planta, de tal manera que una mayor concentración de nutrientes de la hoja verde puede estar correlacionada positivamente con la concentración de nutrientes en la hoja senescente (Lathja 1987; Kobe, Lepczyk y Iyer 2005), aunque en otros casos esto no ha sido así (Pugnaire and Chapin 1993).

La idea de que las especies de suelos menos fértiles sean más eficientes reabsorbiendo nutrientes (Vitousek 1982; Ralhan y Singh 1987) también se ha barajado como un patrón para explicar la reabsorción, aunque no se ha podido demostrar satisfactoriamente (Kobe, Lepczyk y Iyer 2005), encontrándose incluso resultados que contradicen esta teoría (Pugnaire y Chapin III 1993).

La concentración final de nutrientes en la hoja senescente (llamada *nutrient proficiency*, en inglés), también ha sido asociada a algunos rasgos foliares que describen el espectro económico de la hoja (Wright et al. 2004) como la longevidad foliar y la relación de masa foliar por área (LL-LMA; Aerts 1996; Wright y Westoby 2003).

La mayoría de los estudios se centra en la reabsorción del N y P, de forma que los datos que se tienen de la reabsorción del resto de nutrientes sean limitados. Además, los porcentajes de reabsorción son muy variables según la especie y el estudio. La razón por la que unas especies reabsorben más nutrientes que otras no se conoce exactamente. Tampoco se conoce qué efectos puede tener el cambio climático sobre este proceso, lo que hace necesaria la comprensión de los mecanismos que lo rigen, para poder hacer predicciones frente al cambio global.

El objetivo principal de este capítulo (**Capítulo 5**, *Relationships between functional traits in green and senesced leaves and nutrient resorption in 33 woody Mediterranean species*) es conocer los efectos que tienen los rasgos funcionales de la hoja verde sobre las características de la hoja senescente (que determinan la calidad de la hojarasca), y sobre la eficiencia en la reabsorción de nutrientes en 33 especies leñosas. También se pretende encontrar patrones generales de eficiencia en la reabsorción de nutrientes según el tipo funcional de las especies (perennifolias o caducifolias).

Para ello recolectamos hojas verdes y senescentes de 33 especies leñosas (perennifolias y caducifolias) en campo. Una vez en el laboratorio tomamos medidas de características morfológicas y mecánicas de las hojas, como por ejemplo el grosor de la hoja (*thickness*, Thck), la concentración de clorofila (*chlorophyll*, Chlo), la relación de masa por área foliar (LMA), la relación entre el peso del limbo y el peciolo (*limb/petiole*, lmb/pet) o la resistencia a la rotura de la hoja (*toughness*, Tough). Además determinamos la concentración de carbono y nutrientes (N, P y K) en ambas fases de la hoja y calculamos la eficiencia de reabsorción de nutrientes de cada especie, usando como aproximación un factor de corrección que tiene en cuenta la pérdida de peso de la hoja en el proceso de senescencia, considerando hojas con el mismo área. A partir de estos datos podemos buscar rasgos funcionales asociados a la eficiencia en la reabsorción de nutrientes, y observar si existen patrones o estrategias generales según el grupo funcional.

Efectos de la hojarasca sobre el crecimiento de las plantas

La siguiente fase del reciclaje de nutrientes es llevada a cabo por la descomposición de la hojarasca y la liberación de compuestos de ésta al suelo. Es por esto que la hojarasca tiene un papel muy importante en los ecosistemas terrestres (Lambers et al. 2008). Modifica las condiciones del suelo, como la temperatura (Heady 1956; Watt 1974), o el contenido de agua (Zaady et al. 1996; Violle et al. 2006), debido a sus propiedades físicas. Además, también puede tener algunos efectos derivados de su composición química, como modificar el pH del suelo (Reich et al. 2005; Koorem et al. 2011), la liberación de nutrientes (Facelli y Pickett 1991); o su inmovilización, debida a la liberación de de compuestos alelopáticos (Foster y Gross 1998; Bonanomi et al. 2006; Samedani et al. 2013).

Todo esto se traduce en efectos sobre el crecimiento de las plantas. Para cuantificar dichos efectos, suelen usarse variables como la biomasa aérea o de la planta completa (Xiong y Nilsson 1999; Boeken y Orenstein 2001; Dorrepaal et al. 2007; Fisher et al. 2013). Pocos estudios han estimado los efectos sobre el crecimiento de la raíz, y los que lo han hecho los han medido en términos de biomasa o longitud (Bonanomi et al. 2011; Bughio et al. 2013; Pérez-Corona et al. 2013), aunque otros rasgos, como el grosor de la raíz o la longitud específica de raíz (SRL), también pueden ser interesantes, dado que pueden determinar que las raíces tengan distintas funciones (Eissenstat et al. 2000).

El objetivo general de este capítulo (**Capítulo 6**, *Short-term effects of litter from 21 woody species on plant growth and root development*) es comprobar si los rasgos funcionales de la hojarasca como la composición química o la tasa de descomposición pueden explicar o predecir los distintos efectos a corto plazo de la hojarasca de 21 especies leñosas sobre el crecimiento de las plantas y el desarrollo de la raíz. Además pretendemos saber si existen patrones generales de los efectos de la hojarasca según el tipo funcional de la especie de hojarasca (perennifolia o caducifolia). Por último, analizamos la respuesta de la raíz al incremento de la disponibilidad de nutrientes en el suelo.

Para alcanzar nuestros objetivos recogimos hojarasca de 21 especies leñosas (perennifolias y caducifolias) en campo. Se determinó la composición química de una submuestra de la hojarasca fresca de cada especie (C, N, P, K, Na, Ca, Mg y polifenoles totales). Para este capítulo realizamos tres ensayos al mismo tiempo:

- *(i)* *Ensayo de crecimiento con hojarasca:* colocamos hojarasca fresca (una sola especie de hojarasca por maceta) en macetas en la que se cultivó *D. glomerata*.
- *(ii)* *Ensayo de crecimiento con fertilizante:* se añadieron dosis crecientes de fertilizante (nitrógeno y fósforo) como para evaluar la respuesta de nuestra especie *diana* a incrementos en la disponibilidad de nutrientes y también evaluar la respuesta del desarrollo de la raíz en condiciones de escasez de nutrientes.
- *(iii)* *Ensayo de descomposición:* se realizó un experimento de diez semanas para determinar la tasa de descomposición de las diferentes especies de hojarasca en microcosmos.

Puntos fuertes de esta tesis

-Empleo de ensayos multiespecíficos: los resultados de estudios con pocas especies hacen que las generalizaciones a nivel de comunidad sean más difíciles de hacer. El empleo de experimentos con múltiples especies distintas sometidas a las mismas condiciones pueden dar lugar a conclusiones mucho más ricas, y que aporten información más detallada sobre las características de las especies y los procesos de los ecosistemas.

-Exploración de una amplia cantidad de rasgos funcionales: a lo largo de los experimentos de esta tesis se ha hecho un enorme esfuerzo por cuantificar un amplísimo espectro de rasgos vegetales distintos, incluyendo rasgos poco estudiados. Las variables de raíz en invernadero y en campo, junto con características del peciolo, son rasgos poco estudiados por la dificultad que entraña su muestreo.

-Estudios en invernadero y en campo, comparando rasgos en plántulas y adultos: no existen estudios que comparen los rasgos funcionales asociados al crecimiento de plántulas en condiciones controladas con los de adultos en condiciones de campo. Comprobar estas relaciones es de gran importancia para saber si las relaciones son las mismas y por tanto se pueden extrapolar los resultados en condiciones controladas con los resultados en campo.

-Duración del episodio de sequía: no existen muchos experimentos de sequía que se prolonguen hasta observar la muerte de la planta. Esto nos permite extrapolar nuestros resultados a un episodio de sequía prolongada. Esto es muy conveniente, ya que predicciones para el cambio climático proponen una mayor frecuencia e intensidad de los episodios de sequía (Stoker et al., 2013).

-Gran esfuerzo para relacionar información de los distintos procesos de las plantas que abarca esta tesis. En esta tesis se ha hecho un gran esfuerzo por hacer una profunda revisión de distintos procesos de las plantas y unirlos a través de un hilo conductor común: los rasgos funcionales. Esto hace de ella una aportación muy completa al conocimiento del funcionamiento de las plantas y ecosistemas mediterráneos.

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Capítulo 2. Crecimiento de plántulas y árboles de seis especies de *Quercus*

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Crecimiento de plántulas y árboles de seis especies de Quercus

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Resumen

Los objetivos principales de este trabajo son: a) describir algunas características importantes relativas a los rasgos funcionales en plántulas de las especies del género Quercus, b) determinar cómo varía la proporción de hojas, tallo y raíz en adultos, y c) estimar las tasas de crecimiento relativo (RGR) de estas especies para plántulas e individuos adultos. Para ello, usamos los datos de experimentos realizados en plántulas bajo condiciones controladas, del Inventario Forestal Nacional (IFN2 y IFN3) y de las ecuaciones alométricas desarrolladas por Montero et al. (2005). Las especies de Quercus se caracterizan por tener semillas grandes, y las plántulas presentan una baja RGR y área específica foliar (SLA) y una alta proporción de raíz (RMR). Uno de los factores más importantes que explican las diferencias en RGR entre las especies de Quercus (tanto en plántulas como en adultos) fue la diferencia en SLA. Considerando los datos de biomasa de árboles, se observa que la proporción de hoja (LMR) y de raíz (RMR) disminuye con el tamaño, mientras que la proporción de tallo (SMR) aumenta con éste. Si consideramos un árbol medio de 20 cm de diámetro, los valores de LMR son de sólo 1-5% y los de SMR van del 50-80%. Considerando los datos del IFN, dentro de cada especie existe una gran variación en RGR. Aun así, cuando se comparan las RGR de las plántulas con los adultos se observa una relación significativa y positiva. Uno de los factores que afectó significativamente a RGR fue el tamaño del árbol, de forma que árboles de mayor tamaño crecían más lentamente.

Palabras clave: área específica foliar; crecimiento relativo; alcornoque, encina; quejigo; roble

Abstract

Seedling and tree growth of six Quercus species

The main objectives of this work were: a) to describe some important features relative to the functional traits of seedlings of species belonging to the genus Quercus, b) to calculate biomass allocation patterns in leaves, stem and roots in adults, and c) to calculate the relative growth rates (RGR) of these species for seedlings and adults. To do this, we used data of experiments with seedlings under controlled conditions, the National Forest Inventory (IFN2 and IFN3) and the allometric equations developed by Montero et al. (2005). Quercus species were characterized by large seeds, and the seedlings had a low RGR and specific leaf area (SLA) and a high proportion of root (RMR). One of the most important factors explaining differences in growth rates among seedlings and adults of Quercus species were the differences in SLA. Considering data of tree biomass, the proportion of biomass in leaves (LMR) and roots (RMR) decreases with tree size, while the proportion of biomass in stem (SMR) increases with tree size. Considering an average tree with diameter of 20 cm, the values of LMR are only between 1-5% and SMR ranging from 50 to 80%. Considering the IFN data, within each species, the RGR values were highly variable. Still, when the RGR of seedlings were compared with those of adults a significant and positive relationship was observed. One of the factors that significantly affected RGR was tree size, so that bigger trees grew more slowly.

Key words: cork oak; English oak; holm oak; Portuguese oak, relative growth rate (RGR); specific leaf area

Introducción

El crecimiento de las plantas es un proceso complejo, que de forma simplificada se puede describir como el balance entre la captura y las pérdidas de carbono, nutrientes y agua (ver Lambers et al. 1998). Se define el crecimiento como el incremento de biomasa por unidad de tiempo. La importancia de estudiar el crecimiento de las plantas reside en que es la entrada de energía mayoritaria en los ecosistemas y por tanto de la que dependen los demás niveles tróficos. Dado que la energía luminosa asimilada por las plantas se almacena en moléculas de carbono, las plantas ejercen un papel importante en el ciclo de carbono, actuando a la vez como fuente y sumidero (e.g. Dixon et al. 1994). La actividad humana está incrementando exponencialmente la concentración de CO_2 en la atmósfera y existe una gran preocupación por su papel como efecto invernadero y las consecuencias que puede tener en la modificación del clima. Por ello, es importante conocer las fuentes y sumideros de carbono, para limitar en lo posible el incremento de CO2 atmosférico.

Las plantas leñosas que constituyen los bosques y matorrales tienen una gran importancia, ya que estos tipos de formaciones ocupan una gran superficie en la península Ibérica (36 % de la superficie española, Ruíz de la Torre 1990) y a nivel mundial (31 % de la superficie total, FAO 2012). Los bosques de *Quercus* constituyen la mayor parte de la superficie forestal de la península Ibérica (30% del área basimétrica total, Inventario Forestal Nacional, IFN3). En las últimas décadas se ha realizado un enorme esfuerzo para delimitar la estructura, densidad y composición de los bosques de España, gracias a los inventarios forestales (IFN). El uso de los datos del IFN permite obtener datos de crecimiento de árboles en condiciones de campo a escalas espaciales amplias. Existen muy pocos trabajos que hayan estimado las tasas de crecimiento (basadas en biomasa) en campo. Esto es debido principalmente a las dificultades de la estimación de biomasa (principalmente de la raíz) (Poorter et al. 2012). La mayoría de los trabajos estiman el crecimiento basado en incrementos en altura y/o en diámetro del tronco, considerándose que son buenas estimas del crecimiento en biomasa.

En cambio, se han realizado muchos estudios sobre crecimiento en plántulas de Quercus, y cómo distintos factores (luz, agua, nutrientes) pueden afectar su crecimiento (e.g. Quero et al. 2006, Sánchez et al. 2006). Otros estudios (Antúnez et al. 2001, Ruíz-Robleto y Villar 2005, Lopez-Iglesias et al. 2014) han estimado las tasas máximas de crecimiento relativo, y para ello se han realizado ensayos en condiciones controladas (en invernadero, usando niveles de agua y nutrientes óptimos para el crecimiento). Este tipo de metodología es necesaria cuando se realizan estudios comparativos, ya que todas las especies deben estar en las mismas condiciones, para que otros factores no influyan en las variables medidas. Sin embargo, también hay que reconocer que los valores obtenidos en condiciones controladas y en plántulas podrían diferir de aquellos medidos en condiciones de campo y en adultos. No existen trabajos que comparen las tasas de crecimiento de distintas especies de Quercus en condiciones controladas y en campo.

Cuando se habla de crecimiento se pueden dar estimas de crecimiento absoluto o relativo (Paine et al. 2012). El crecimiento absoluto es el incremento de biomasa por tiempo, y puede venir dado en kg $año^{-1}$. La tasa de crecimiento relativo (RGR, relative growth rate) es el incremento de biomasa por unidad de biomasa y tiempo, y puede expresarse en las unidades mg g^{-1} $año^{-1}$. En general, se acepta que para fines comparativos es más idóneo usar RGR ya que así se elimina el artefacto del tamaño de la planta. Una planta grande crece más rápido (en valor absoluto, g $día^{-1}$) que una planta pequeña, por el sólo hecho de ser más grande, aunque la eficiencia por unidad de biomasa (RGR) pueda ser menor (Poorter y Garnier 1999). Los valores de crecimiento absoluto; sin embargo, pueden ser particularmente interesantes desde el punto de vista del papel de las plantas en el ciclo del carbono.

La gran variación en RGR entre las distintas especies ha llevado a preguntarse cuáles son las causas de esta gran variación. Esto ha llevado a descomponer RGR en dos factores principales: la razón de área foliar (LAR, leaf area ratio, relacion del área de la planta con respecto a la biomasa) y la tasa de asimilación neta (NAR, net assimiation rate, ganancia de biomasa por unidad de área y tiempo) (Poorter y Remkes 1990). De esta forma, RGR = LAR × NAR). A su vez, LAR se puede descomponer en el área específica foliar (SLA, specific leaf area, relación del área foliar con respecto a biomasa foliar) y la proporción de hoja (LMR, leaf mass ratio). Así, LAR = SLA × LMR. Aunque hay estudios en las que el componente fisiológico (NAR) tiene más importancia en la variación de RGR, la mayoría de los estudios concluyen que el factor morfológico (LAR) es más importante. Además, muchos estudios han llegado a la conclusión de que una variable muy sencilla de medir, como es el área específica foliar (SLA) es un buen predictor de las tasas máximas de RGR (Poorter y Remkes 1990, Garnier 1992, Lambers y Poorter 1992, Antunez et al. 2001).

También, otras características como la proporción de hojas (LMR) o la concentración de nitrógeno foliar (N), se han descrito como características que explican la variación en RGR (Huante et al. 1995, Cornelissen et al. 1996, Villar et al. 2006, Villar et al. 2008). Estas características (morfológicas, fisiológicas y/o fenológicas) de las especies que influyen directa o indirectamente al crecimiento, la reproducción y la supervivencia se denominan se denominan en sentido amplio "rasgos funcionales" (sensu Violle et al. 2007).

Los objetivos de este trabajo son: (1) conocer qué diferencias existen en rasgos funcionales entre las especies de *Quercus* y otras especies mediterráneas, (2) conocer los patrones de inversión de la biomasa en hojas, tallo y raíz en las especies de *Quercus* en condiciones de campo; y (3) determinar las tasas de crecimiento en altura, diámetro y biomasa en especies de *Quercus* en adultos y en condiciones de campo. Para ello, se han usado datos obtenidos en condiciones controladas (en invernadero) y datos de los Inventarios Forestales Nacionales (IFN2 y IFN3) combinados con las ecuaciones alométricas desarrolladas por Montero et al. (2005). Con todos estos datos se han calculado las tasas de crecimiento relativo en seis especies de *Quercus*.

Material y métodos

Rasgos funcionales y crecimiento relativo en condiciones controladas

Los datos de rasgos funcionales y tasas de crecimiento relativo se obtuvieron de dos experimentos realizados en condiciones controladas, cuyos datos están parcialmente publicados (Antúnez et al. 2001; Ruiz-Robleto y Villar 2005; Villar et al. 2008). Los dos experimentos realizados en condiciones controladas (invernadero) tuvieron unas condiciones de cultivo y desarrollo similar. La lista de especies (siete especies de *Quercus* y 17 especies leñosas) se puede consultar en Villar et al. (2008). En el presente trabajo se han incluido los siguientes rasgos funcionales: peso fresco de semilla (g), área específica foliar (SLA, m^2 kg^{-1}), concentración de nitrógeno foliar (N), tasa de crecimiento relativo (RGR, mg g^{-1} $día^{-1}$), razon de área foliar (LAR, m^2 kg^{-1}), tasa de asimilación neta (NAR, g m^{-2} dia^{-1}) y proporción de raíces (RMR, root mass ratio, %) y hojas (LMR, leaf mass ratio, %). De forma resumida se describe a continuación la metodología. Previo a la siembra, se tomó el peso fresco de las semillas de una submuestra. Las semillas se pusieron a germinar en macetas de 3.5 L y con un sustrato formado por arena y turba (3:1). Las macetas se abonaron con un abono de liberación lenta y se regaron periódicamente para asegurar un crecimiento óptimo. Se realizaron varias cosechas para determinar el peso seco de hojas, tallo y raíz. Con el peso total se calculó la tasa de crecimiento relativo como RGR = [Ln (peso t2) – Ln (peso t1)] / (t2 –t1), siendo t2 y t1 los tiempos de la cosecha final e inicial, respectivamente. El área específica foliar se calculó como el área foliar respecto al peso seco foliar. Para más detalles se puede consultar Villar et al. (2008).

Crecimiento relativo en condiciones de campo

Los datos observacionales de crecimiento para individuos adultos se obtuvieron del IFN de España. El IFN es una base de datos nacional de muestreos periódicos sistemáticamente distribuidos cada kilómetro a lo largo de toda el área forestal de España (Villaescusa y Díaz 1998; Villanueva 2004). Las parcelas del IFN tienen radio variable según el diámetro normal (dbh) de los árboles medidos y su localización en las parcelas. Las parcelas inventariadas durante el segundo IFN (IFN2, en los años 1986-96), fueron revisitadas durante el tercer IFN (IFN3, 1997-2007), es decir, con un periodo medio entre ambos muestreos de 11 años. De cada individuo se registra la especie, y se toman medidas de su altura y diámetro normal. Al estar todos los árboles identificados individualmente, es posible calcular una tasa de crecimiento diametral y en altura a nivel de individuo. Dado que las parcelas cubren todo el territorio nacional, las condiciones ambientales de las parcelas son muy distintas en cuanto a temperatura, precipitación y tipo de suelo. Por ejemplo, la temperatura media anual para las parcelas con presencia de especies del genero Quercus varía entre los 4 y 18 ºC, y la precipitación anual va desde 200 mm (Almería) a 2500 mm (Galicia), aunque estas condiciones son también muy variables dependiendo de la orografía y la continentalidad.

Para este estudio se seleccionaron individuos de seis especies del género *Quercus (Q. canariensis, Q. faginea, Q. ilex, Q. pyrenaica, Q. robur y Q. suber)*, que cumplieran los siguientes criterios: (i) que fueran árboles vivos en parcelas permanentes comparables individuo a individuo; (ii) que su diámetro normal en el IFN2 fuera mayor de 200 mm y que sus cambios en dbh y altura entre inventarios consecutivos fueran mayores o iguales a 0; (iii) que fueran parcelas no manejadas, sin evidencias de corta o aclareo y sin evidencias de ser plantaciones; y (iv) que el área basal de la especie en cuestión fuera mayor del 50% del área basal total en la parcela. Siguiendo estos criterios obtuvimos datos de 548 individuos distribuidos en 171 parcelas para *Q. canariensis*; 3016 individuos distribuidos en 1121 parcelas para *Q. faginea*; 20084 individuos distribuidos en 6915 parcelas para *Q. ilex*; 7611 individuos distribuidos en 1709 parcelas para *Q. pyrenaica*; 5182 individuos distribuidos en 1115 parcelas para *Q. robur* y 9633 individuos distribuidos en 2288 parcelas para *Q. suber.* Las fuertes diferencias en el número de árboles y de parcelas por especie, especialmente de *Q. canariensis* con un bajo número de árboles, podría suponer un sesgo en los datos obtenidos para esta especie, como se comentará más adelante.

Calculo de variables y análisis estadísticos

La comparación de los rasgos funcionales de las especies de *Quercus* y otras especies leñosas obtenidos para plántulas en condiciones controladas se hizo con el test no paramétrico de Kruskal-Wallis.

Para conocer los valores medios de la proporción de la biomasa en hojas, tallo y raíz (LMR, SMR y RMR, respectivamente) de las especies de *Quercus*, se usaron los datos de Montero et al. (2005). Estos datos corresponden a la biomasa predicha para hojas, tallo y raíz usando ecuaciones alométricas en función de las clases diamétricas comprendidas entre 5 y 70 cm de dbh, (clases diamétricas de 5 cm). En la Tabla 2 (pg. 35) de Montero et al. (2005) se detallan las ecuaciones usadas para cada especie y fracción.

Para estimar la biomasa total (parte aérea y radical) de los árboles individuales de cada parcela aplicamos las ecuaciones alométricas de Montero et al. (2005), que relacionan la biomasa seca total del árbol y sus fracciones (i.e. hojas, tallo y raíz) en función del diámetro. A pesar de las limitaciones de estas ecuaciones, ya que no tienen en cuenta que la asignación de biomasa a los distintos órganos de la planta pueden estar afectados por la variabilidad ambiental, esta aproximación cumple, a nuestro juicio, con el objetivo principal de comparación entre las seis especies de *Quercus*. Por otro lado, no existen datos de estas especies sobre cómo se modifica la asignación a hojas, tallo y raíz en función de variables ambientales.

Con los datos procedentes de los dos inventarios (IFN2 y IFN3, calculamos la tasa de crecimiento relativo (RGR) de cada individuo de tres maneras distintas: (i) como incremento en altura (RGRalt , m m^{-1} $año^{-1}$); (ii) incremento de diámetro (RGRdbh , cm cm^{-1} $año^{-1}$); e (iii) incremento de biomasa (RGRbiomasa, mg g^{-1} $año^{-1}$). La estima de RGR se realizó usando la siguiente fórmula: RGR = [Ln (X2) – Ln (X1)]/ tiempo, siendo X la variable con la que se estima RGR (siendo altura, diámetro o biomasa), los subíndices 2 y 1 se refieren a las medidas en el los tiempos 2 y 1 y el tiempo los años entre 2 y 1 (en general 10 años). Los datos de SLA y N foliar de individuos en campo fueron tomados de Wright et al. (2004) y fuentes propias (De la Riva et al. 2014).

Los análisis estadísticos (promedios por especie y correlaciones entre variables) se llevaron a cabo con el programa Statistica 7.1 (StatSoft, Inc., USA). Las ecuaciones de regresión para estimar la relación entre RGR y tamaño del árbol se calcularon utilizando distintas funciones con el programa Statgraphics 5.1 (Statistical Graphics Corp., USA).

Resultados

Comparación de rasgos funcionales entre Quercus y otras especies leñosas en plántulas bajo condiciones controladas

Las especies de *Quercus* difieren significativamente (P < 0.01) en una serie de rasgos funcionales con respecto a otras especies leñosas de ecosistemas mediterráneos. Las especies de *Quercus* se caracterizan por tener semillas grandes, bajas tasas de crecimiento relativo (RGR) y área específica foliar (SLA) y una alta proporción de raíz (RMR) (Fig. 1). Además, cabe remarcar que para algunas variables, la variabilidad respecto a la media fue alta en el grupo de *Quercus* (ver Fig. 1), similar al grupo del resto de especies que comprenden varios géneros y familias distintas.

Uno de los factores más importantes que explicaron las diferencias en RGR entre las especies de *Quercus* fue el área específica foliar (SLA), presentando una relación cercana a la significación (R2 = 0.55, P = 0.06; Fig. 2 A). La relación entre RGR y SLA sí fue significativa para las otras especies leñosas, aunque con un R^2 muy similar (0.55). En relación a los componentes morfologicos (LAR) y fisiológicos (NAR) de RGR, se observó que RGR dependió más de NAR que de LAR para los *Quercus*, en cambio, para las otras especies fue al contrario (Fig. 2 B y C). La proporción de hojas

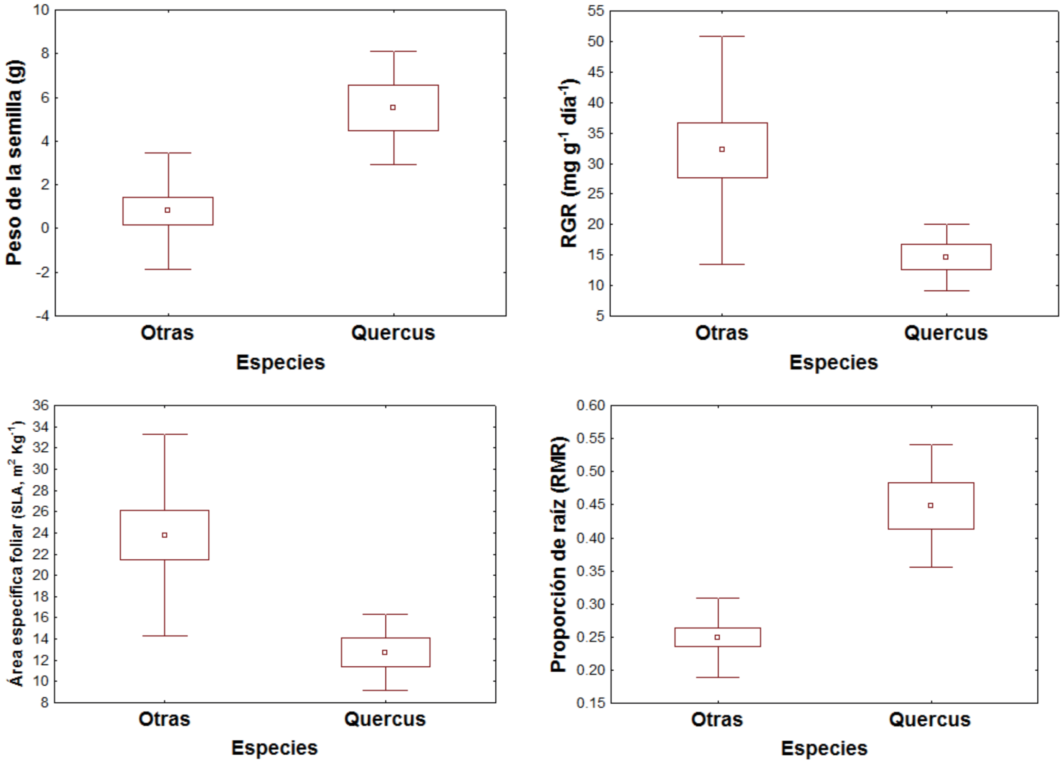

Figura 1. Valores medios ± error estándar (caja) y desviación estándar (patas) de peso de semilla fresco, tasa de crecimiento relativo (RGR), área específica foliar (SLA) y proporción de raíz (RMR) de las especies de Quercus (siete especies) y otras especies leñosas mediterráneas (17 especies), medidas bajo condiciones controladas y en plántulas de un año. Ver Villar et al. (2008) para la lista completa de especies en ambos grupos.

Figure 1. Mean values ± standard error (box) and standard deviation (whisker) for fresh seed mass, relative growth rate (RGR), specific leaf area (SLA) and root mass ratio (RMR) of Quercus species (seven species) and other Mediterranean woody species ("Otras", 17 species), measured under controlled conditions and in seedlings of 1 year. See Villar et al. (2008) for a complete list of species in both groups.

(LMR) y de raíz mostraron una fuerte relación negativa para los Quercus (Fig. 2 D).

Distribución de biomasa y tasas de crecimiento de Quercus en condiciones de campo

En relación a la proporción de hoja, tallo y raíz se observa, en general, que la proporción de hoja (LMR) y de raíz (RMR) disminuye con el tamaño del árbol, mientras que la proporción de tallo (SMR) aumenta con el tamaño del árbol (Fig. 3). Aun así, es de destacar, que la dinámica de algunas especies es distinta. Por ejemplo, en relación a la proporción de hoja, hay un descenso de LMR con el tamaño de árbol muy acusado para *Q. faginea*, mientras que éste es menor para *Q. canariensis*. En cuanto a la proporción de tallo, también se observan diferencias entre especies. Todas aumentan SMR con el tamaño de árbol, salvo *Q. canariensis*, que lo reduce. En relación a la proporción de raíz, de forma similar, *Q. canariensis* presenta un comportamiento un tanto atípico, ya que aumenta el valor de RMR con el tamaño del árbol. Si consideramos un árbol medio de 20 cm de diámetro, los valores de LMR están comprendidos entre el 1-5%, los de SMR entre el 50-80% y los de RMR entre el 20-40% (Fig. 3).

Las tasas de crecimiento pueden venir expresadas en términos de altura, diámetro o biomasa. A pesar de haber una relación significativa ($P < 0.0001$) entre RGRdbh y RGRalt para todas las especies, salvo *Q. canariensis* ($P = 0.08$), el porcentaje de variación de RGRdbh. explicado por RGRalt fue muy bajo (entre el 1.2 y el 2%) (Fig.

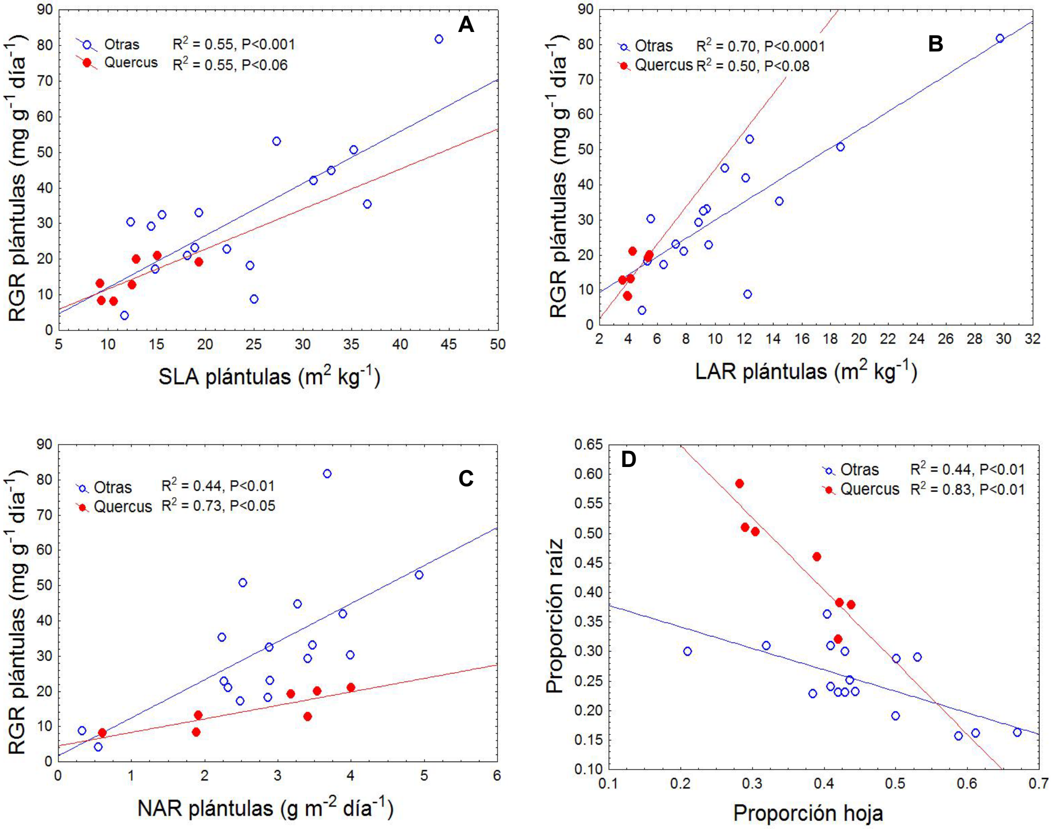

Figura 2. Relación entre la tasa de crecimiento relativo (RGR) con **(A)** el área específica foliar (SLA), **(B)** la razón de área foliar (LAR) y **(C)** la tasa de asimilación neta (NAR). **(D)** Relación entre la proporción de raíz y de hoja en 7 especies de *Quercus* y otras especies leñosas mediterráneas (Otras, 17 especies) medidas en condiciones controladas y en plántulas de 1 año.

Figure 2. Relationship between relative growth rate (RGR) with **(A)** specific leaf area (SLA), **(B)** the leaf area ratio (LAR) and **(C)** the net assimilation rate (NAR). **(D)** Relationship between the proportion of root and leaf in 7 species of *Quercus* and other Mediterranean woody species (Otras, 17 species) measured under controlled conditions and in seedlings of 1 year.

4, R2 = 0.014, para *Q. pyrenaica*). Dado que los datos en términos de biomasa se calculan en base al diámetro, la correspondencia de los datos de RGRdbh y RGRbiomasa es exactamente la misma.

Las tasas de crecimiento en campo son muy variables dentro de una misma especie. Usando un diagrama de frecuencias se ha representado el porcentaje de individuos de cada especie con unos intervalos determinados de RGR (Fig. 5). Uno de los factores que afectó significativamente ($P < 0.001$) al RGR en todas las especies fue el tamaño del árbol, de forma que árboles de mayor diámetro crecían más lentamente (Fig. 6).

A pesar de la alta variabilidad en RGR (Fig. 5 y 6), podemos estimar unos valores medios por especie. Para ello, se ha considerado la categoría diamétrica de 20-40 cm de dbh, que es la que muestra los valores máximos de RGR. El ranking de RGR (mg g^{-1} año^{-1}) para las seis especies consideradas fue: *Q. ilex* (16.1), *Q. faginea* (20.7), *Q. suber* (21.2), *Q.pyrenaica* (23.4), *Q. canariensis* (24.7), *Q. robur* (29.5).

Cuando se compararon las tasas de crecimiento de las plántulas con los adultos (medidas en las clases diamétricas de 20-40 cm) se observó una relación positiva y

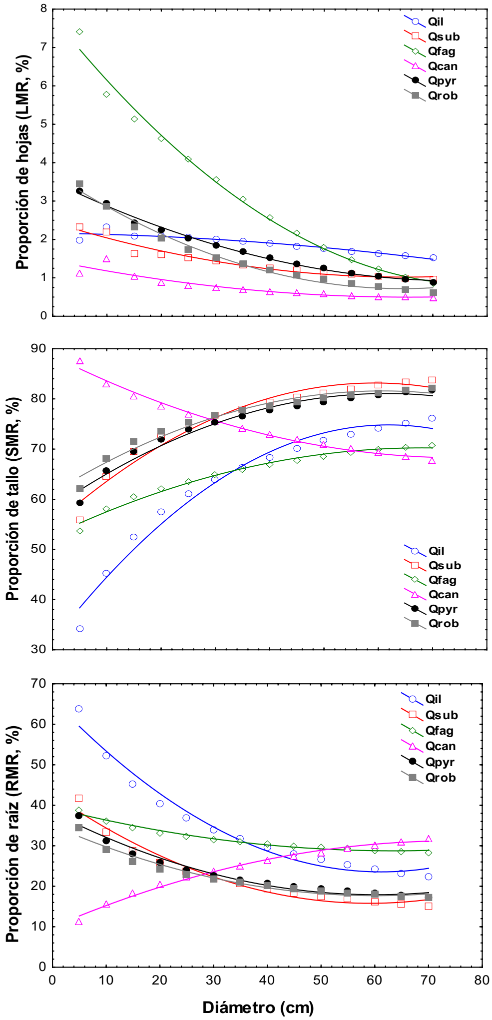

Figura 3. Variación de la proporción de hoja (LMR), tallo (SMR) y raíz (RMR) con el diámetro del árbol para seis especies de *Quercus*. Datos obtenidos de Montero et al. (2005).

Figure 3. Variation in the proportion of leaf (LMR), stem (SMR) and root (RMR) with tree diameter for six species of *Quercus*. Data from Montero et al. (2005).

significativa para los valores medios de las seis especies consideradas (Fig. 7 A). Las tasas de crecimiento en campo estuvieron correlacionadas significativamente ($P < 0.01$) con el SLA y la concentración de nitrógeno foliar (Fig. 7 B y C).

Discusión

Comparación de rasgos funcionales entre *Quercus* y otras especies leñosas

Las especies del género *Quercus* difieren en una serie de rasgos funcionales claves con respecto a otras especies leñosas. Una de las diferencias importantes fue el peso de la semilla, puesto que las especies de *Quercus* presentan semillas que fueron seis veces mayores (5.5 ± 1 g peso fresco, media ± desviación estandar) al de las otras especies leñosas con las que coexisten (0.8 ± 0.6 g). El peso de la semilla determina las reservas que tiene la plántula al germinar. Un peso de semilla grande presenta ciertas ventajas durante los primeros estadios de la plántula ya que le permite soportar situaciones con una baja ganancia de carbono, como por ejemplo en condiciones de sombra (Quero et al. 2007). Una semilla grande también origina plántulas con una biomasa mayor (Quero et al. 2007, Góndález-Rodríguez et al. 2010, Perez-Ramos et al. 2010), lo que puede de conferirle ventajas competitivas como el acceso a recursos limitantes (e.g. agua, nutrientes, luz).

Las especies del género *Quercus* suelen presentar tasas de crecimiento relativamente bajas (14.6 ± 2.0 mg g^{-1} día^{-1}), frente a otras especies leñosas en las que se observaron mayores tasas de crecimiento relativo (32.2 ± 4.5 mg g^{-1} día^{-1}). Los bajos valores de RGR podrían deberse a que las especies de Quercus presentan hojas con un valor bajo de área específica foliar (SLA), que las hace ser especies de tipo conservativo (Diaz et al. 2004, Wright et al. 2004). En general, en muchos estudios se ha encontrado que RGR está relacionado positivamente con SLA, tanto para especies herbáceas (Garnier 1992; Lambers and Poorter 1992) como en especies leñosas (Reich y Walters 1992; Huante et al. 1995; Cornelissen et al. 1996, Antunez et al. 2001, Ruiz-Robleto y Villar 2005). Las especies de Quercus tambien tienen una baja razón de área foliar (LAR) y una baja tasa de asimilacion neta (NAR). Lo cual se debe en parte a que las especies de Quercus presentan una gran proporción de biomasa en las raíces (45.0 ± 3.0%) frente a otras especies leñosas (25.0 ± 1.4%). Una mayor proporción de raíz puede ser una ventaja para resistir la sequía en climas mediterráneos, al tener acceso a una mayor cantidad de agua en el suelo, lo que puede conferir unas mayores tasas de supervivencia (Lloret et al. 1999). Esta fuerte inversión en raíz hace que la inversión en hojas sea muy baja comparativamente a otras especies.

Por otro lado, dentro de las especies de *Quercus*, existe una variabilidad en sus rasgos funcionales (ver

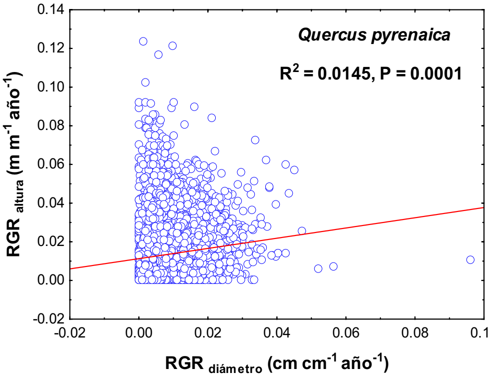

Figura 4. Relación entre la tasa de crecimiento en altura (RGRaltura) y en diámetro (RGRdiámetro) para la especie *Quercus pyrenaica*. Las otras cinco especies de *Quercus* muestran relaciones similares (no mostradas).

Figure 4. Relation between relative growth rate based on height (RGRaltura) and diameter (RGRdiámetro) for the species *Quercus pyrenaica*. The other five *Quercus* species show similar relationships (not shown).

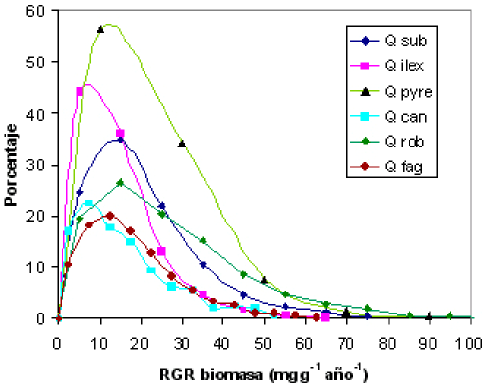

Figura 5. Distribución de los intervalos de las tasas de crecimiento (RGRbiomasa) en las seis especies de *Quercus* a partir de datos observacionales del Inventario Forestal Nacional y las ecuaciones de Montero et al. (2005).

Figure 5. Distribution of intervals of growth rates (RGRbiomasa) in six species of *Quercus* from observational data from the Spanish National Forest Inventory and the equations of Montero et al. (2005).

también la variabilidad respecto a la media en Fig. 1).

En este número especial sobre *Quercus*, el estudio de De la Riva et al. (2014) muestra que en las especies de *Quercus* de la península Ibérica existe un síndrome económico de rasgos funcionales. Por un lado hay especies de carácter conservador (denominadas de tipo conservativo), que presentan, lento crecimiento pero son tolerantes a estreses ambientales como la sequía, como por ejemplo es el caso de *Q. ilex* que presenta hojas con bajo SLA y raíces con bajo área específica radicular (SRA, specific root area), baja concentración de clorofila y nitrógeno en la hoja y alta densidad de la madera. En el otro extremo, se encuentran las especies con mayor capacidad de crecimiento (denominadas de tipo adquisitivo) pero con una menor tolerancia a estreses ambientales, como es el caso de *Q. canariensis*. Este patrón concuerda de forma general con las diferencias entre perennifolias y caducifolias y en la longevidad foliar entre especies de *Quercus* (Antunez et al. 2000).

Distribución de biomasa y tasas de crecimiento en árboles adultos

En relación a los datos de Montero et al. (2005) para árboles adultos en condiciones de campo, podemos observar la baja proporción de hoja que presentan los árboles (para un árbol de 20 cm de diámetro, los valores de LMR oscilan entre 1-3%). Además, esta proporción disminuye con el tamaño del árbol para todas las especies de *Quercus*. Poorter et al. (2012) en una amplia revisión con numerosas especies arbóreas, también encontró una dinámica parecida, con un porcentaje muy bajo de hoja y con una disminución casi lineal con el tamaño de la planta. Es de destacar cómo la proporción de tallo es muy alta (en torno al 70%) y cómo ésta aumenta con el tamaño del árbol. En relación a la proporción de raíz, por término medio es de un 30% (para árboles de 20 cm de diámetro) y esta proporción disminuye ligeramente con la edad. Poorter et al. (2012) también encontró un patrón similar tanto en la proporción del tallo como de la raíz en otras especies leñosas. La dinámica que presenta *Q. canariensis* en relación a la proporción de tallo y raíz es una excepción a la encontrada en el resto de especies de este género. Sólo en esta especie, la proporción de tallo disminuye con el tamaño y la proporción de raíz aumenta con el tamaño de la planta. Habría que comprobar esta dinámica con más datos para ver si es una dinámica atípica de esta especie, o si se debe a otros factores. Por ejemplo, podría ser debido a que en el análisis se han considerando arboles procedentes de masas con estructuras de clases de edad y diámetros muy dispares, que podrían haber afectado de forma diferente a las relaciones de diámetro y las proporciones de hoja, tallo y raíz.

La estima de crecimiento en árboles adultos y en condi-

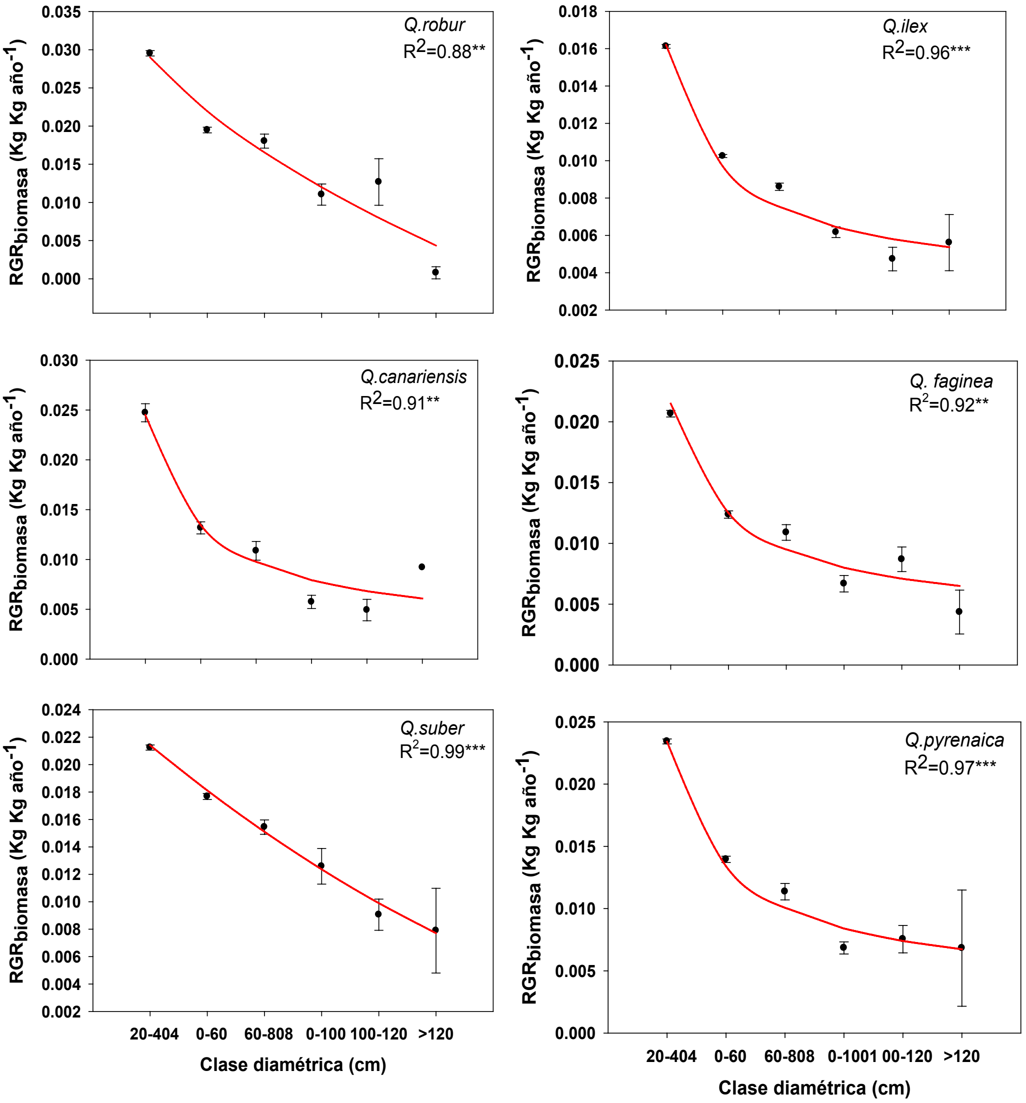

Figura 6. Variación entre la tasa de crecimiento relativo (RGRbiomasa) y el diámetro del tronco (dbh, cm) para árboles adultos de las seis especies de *Quercus*, obtenidos a partir de datos observacionales del Inventario Forestal Nacional y las ecuaciones de Montero et al. (2005). Se representan los valores medios por las clases diamétricas de 20-40; 40-60; 60-80; 80-100; 100-120 y > 120 cm de dbh.

Figure 6. Variation between relative growth rate (RGRbiomasa) and stem diameter (dbh, cm) for adult trees of six species of *Quercus*, obtained from observational data from the Spanish National Forest Inventory and the equations of Montero et al. (2005). Mean values for diameter classes are rendered: 20-40; 40-60; 60-80; 80-100; 100-120 and > 120 cm dbh.

ciones de campo es relativamente complicada. Para medir el crecimiento, la unidad de medida debería ser la biomasa, pero dadas las dificultades para la obtención de datos de biomasa de hojas, tallo y especialmente de raíz, no existen

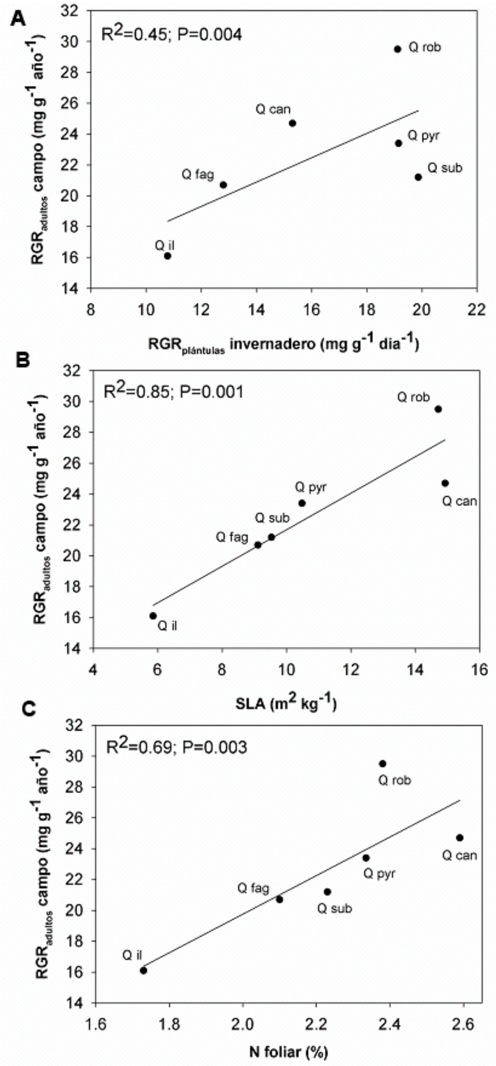

Figura 7. Relación entre la tasa de crecimiento de individuos adultos medidos en condiciones de campo (mg g^{-1} año^{-1}) con respecto a: (A) la tasa de crecimiento relativo medido en condiciones controladas y en plántulas (mg g^{-1} día^{-1}), (B) el área específica foliar (SLA, m^{2} kg^{-1}), y (C) la concentración de nitrógeno foliar (%). Los datos de SLA y N foliar proceden de individuos en campo (tomados de Wright et al. 2004 y fuentes propias, De la Riva et al. 2014). Nótese la diferencia de las unidades de RGR de adultos (mg g^{-1} año^{-1}) y de plántulas (mg g^{-1} día^{-1}).

Figure 7. Relationship between the growth rate of adult individuals measured under field conditions (mg g^{-1} yr^{-1}) with respect to: (A) the relative growth rate measured under controlled conditions and in seedlings (mg g^{-1} day^{-1}), (B) specific leaf area (SLA, m^{2} kg^{-1}), and (C) leaf nitrogen concentration (%). SLA data and N are from individuals foliar field (taken from Wright et al. 2004 and own sources, De la Riva et al. 2014). Note the difference in units of RGR for adults trees (“adultos”) (mg g-1 yr-1) and seedling (“plántulas”) (mg g^{-1} day^{-1}).

muchos estudios observacionales que midan el crecimiento real en biomasa (i.e. medir la biomasa requiere medir el peso de las diferentes fracciones, ver e.g. Montero et al. 2005). Por ello, se usan medidas no destructivas, más prácticas en campo, como por ejemplo la altura y el diámetro del árbol. Estas variables se han medido de forma sistemática en los Inventarios Forestales Nacionales, de forma que comparando inventarios consecutivos podemos obtener estimas individuales de tasa de crecimiento basado en altura y en diámetro. Se podría esperar que las tasas de crecimiento expresadas en una u otra dimensión fuesen similares. Sin embargo, la relación entre el crecimiento relativo en altura y en diámetro fue muy baja. A pesar de que para todas las especies se encontró una relación positiva y significativa, el porcentaje de explicación de una variable sobre otra es muy bajo (del 1 al 2% dependiendo de la especie). Esto sugiere que hay grandes diferencias entre el crecimiento en altura y diámetro, y que por tanto las conclusiones basadas en crecimiento en altura pueden diferir de las basadas en diámetro. Esto podría estar determinado en parte por la competencia, especialmente de la luz, o por la importancia de las condiciones climáticas en la península Ibérica determinando distintas relaciones alométricas en árboles (e.g. Lines et al. 2012), o por un diferente manejo de los árboles, especialemente de aquellos que se encuentran en sistemas agrosilvopastorales, o bien por efecto de una diferente estructura de la masa sobre la relación altura-diámetro. De forma que algunos individuos podrían invertir más en altura, notándose por tanto un menor crecimiento en diámetro y viceversa. Por otro lado, la precisión en la estima de la altura en especies de copas irregulares hacen del cálculo de la RGRalt un parámetro con un grado de error muy superior al de RGRdbh. En resumen, un árbol puede crecer presentando un fuerte incremento en altura pero cambiando poco el diámetro (ver Fig. 4), lo cual, según los predictores de Montero et al. (2005) indicarían un crecimiento nulo. Por ello, nuestros resultados indican la importancia de estimar la biomasa considerando tanto incrementos de altura como en diámetro, y que además podrían variar de una manera importante dependiendo de las condiciones climáticas y estructurales de la parcela.

Las tasas de crecimiento en biomasa están basadas en incremento de diámetro (ya que la biomasa se calculó a partir del dbh, Montero et al. 2005). El crecimiento relativo de árboles estuvo muy relacionado con el crecimiento relativo de las plántulas (Fig. 7 A), aunque hay que resaltar la diferencia de unidades: en plántulas de 10 a 20 mg g^{-1} dìa^{-1} y en arboles de 16 a 30 mg g^{-1} año^{-1}, debido posiblemente a la limitación de recursos que se encuentra en condiciones naturales. Es de destacar también como dos rasgos funcionales importantes de la hoja como el SLA y la concentración de N presentaron una relación muy buena (R^2 de 0.69 a 0.85) con el crecimiento de los árboles en condiciones naturales y podrían considerarse como buenos predictores de las tasas de crecimiento (ver e.g. Ruiz-Benito et al. 2014).

Aún así, dentro de cada especie del genero Quercus las tasas de crecimiento son muy variables, de forma que hay un amplio solapamiento entre todas las especies consideradas (Fig. 5). Esta gran variabilidad puede ser debida a un gran número de factores que pueden afectar, desde factores abióticos (e.g. temperatura, precipitación, disponibilidad de nutrientes) hasta factores bióticos (e.g. competencia, herbivoría, manejo) (ver e.g. Gómez-Aparicio et al. 2011). Sin embargo, los valores medios para cada especie corresponden bastante bien con los datos obtenidos en condiciones controladas para plántulas. Por ello, podríamos concluir que las diferencias encontradas entre las especies de *Quercus* son generalizables, y reales. El crecimiento de cada especie de *Quercus descendió* al incrementarse el tamaño del árbol, de una forma similar al encontrado en otros estudios (Ryan et al. 2004, Coates et al. 2009, Gómez-Aparicio et al. 2011). Ryan et al. (2004) encontró que el descenso en producción de bosques de *Eucalyptus* con la edad fue debido al efecto combinado de un descenso en la fotosíntesis y una mayor inversión en raíces y en la respiración foliar. Nuestros resultados sugieren que el descenso en crecimiento al aumentar el tamaño del árbol podría estar causado por una disminución en la proporción de hoja y que, por tanto, determinaría que proporcionalmente hay una menor superficie foliar para realizar fotosíntesis, así como al incremento de biomasa dedicada a tallo.

Conclusiones

Las especies de Quercus se caracterizan por tener semillas grandes, y las plántulas presentan un bajo crecimiento relativo, una baja área específica foliar y una alta proporción de raíz. Uno de los factores más importantes que explican las diferencias en crecimiento relativo entre las especies de Quercus, tanto en plántulas como en adultos fue las diferencias en el área específica foliar. Considerando los datos de biomasa en condiciones de campo, se observó que la proporción de hoja y de raíz disminuye con el tamaño del árbol, mientras que la proporción de tallo, aumenta con éste. Dentro de cada especie del género *Quercus* existe una gran variabilidad en las tasas de crecimiento relativo a lo largo de la península Ibérica. Aun así, cuando se comparan los crecimientos relativos de las plántulas con los adultos se observa una relación positiva. Uno de los factores que afectó significativamente a las tasas de crecimiento fue el tamaño del árbol, de forma que árboles de mayor tamaño crecían más lentamente, lo cual podría estar determinado por un descenso en la proporción de hojas con el tamaño del árbol.

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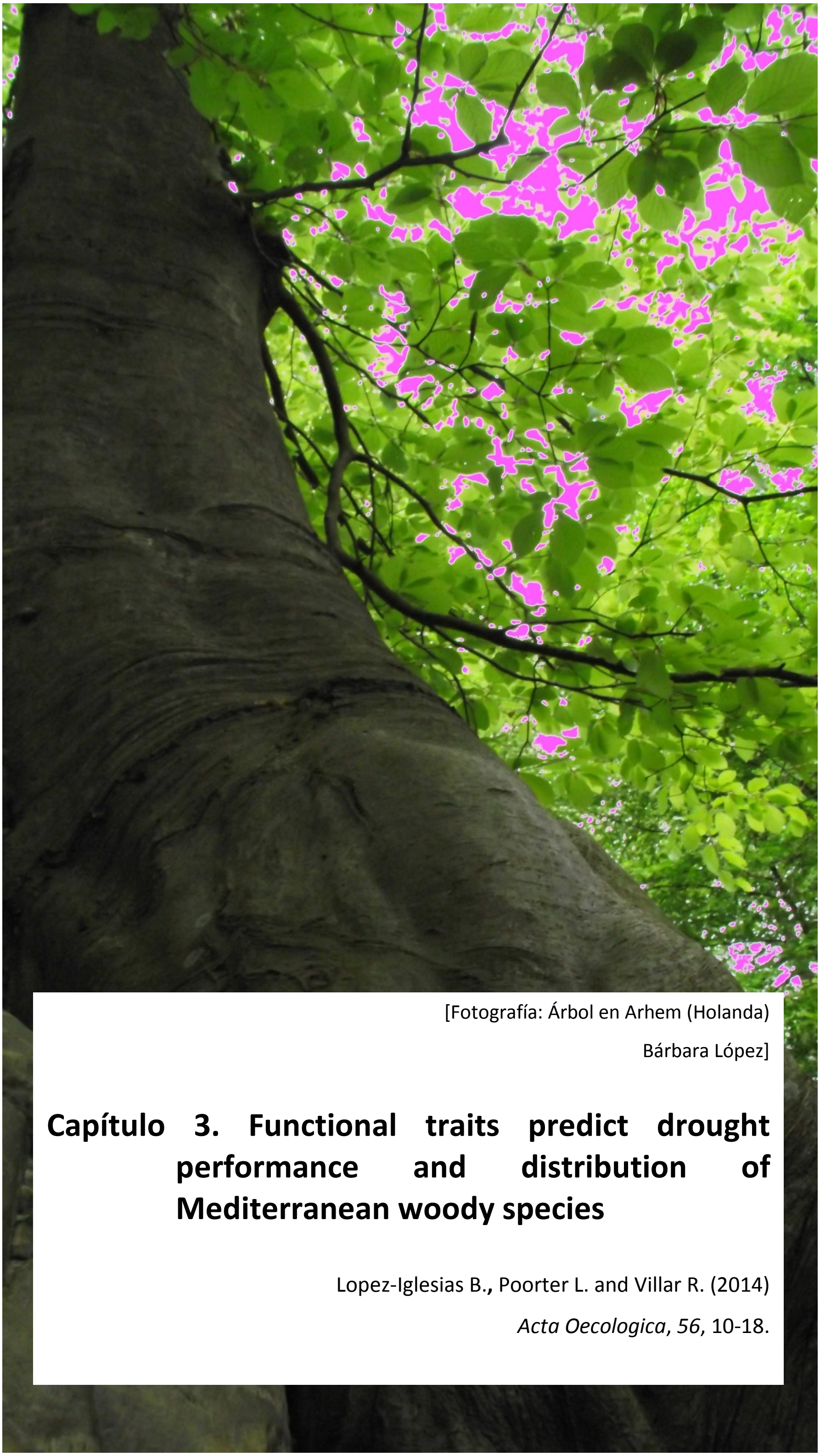

[Fotografía: Árbol en Arhem (Holanda)
Bárbara López]

Capítulo 3. Functional traits predict drought performance and distribution of Mediterranean woody species

Lopez-Iglesias B., Poorter L. and Villar R. (2014)

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Functional traits predict drought performance and distribution of Mediterranean woody species

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ABSTRACT

Water availability is one of the key environmental factors that affect plant establishment and distribution. In many regions water availability will decline with climate change, exposing small seedlings to a greater likelihood of drought. In this study, 17 leaves, stem, root, and whole-plant traits of ten woody Mediterranean species were measured under favourable growing conditions and seedling drought survival was evaluated during a simulated dry-down episode. The aims of this study were: i) to assess drought survival of different species, ii) to analyse which functional traits predict drought survival time, and iii) to explain species distribution in the field, based on species drought survival and drought strategies. Drought survival time varied ten-fold across species, from 19 to 192 days. Across species, drought survival was positively related to the rooting depth per leaf area, i.e., the ability to acquire water from deeper soil layers while reducing transpiring leaf area. Drought survival time was negatively related to species ability to grow quickly, as indicated by high relative growth and net assimilation rates. Drought survival also explained species distribution in the field. It was found that species were sorted along a continuum, ranging between two contrasting species functional extremes based on functional traits and drought performance. One extreme consisted of acquisitive fast-growing deciduous species, with thin, soft metabolically active leaves, with high resource use and vulnerability to drought. The opposite extreme consisted of conservative slow-growing evergreen species with sclerophyllous leaves, deep roots, a low transpiring area, and low water use, resulting in high drought survival and drought tolerance. The results show that these drought strategies shape species distribution in this Mediterranean area.

1. Introduction

Water availability is a key factor for plants in many environments as reported for temperate deciduous forests (Sack, 2004), arid and semi-arid regions (Padilla and Pugnaire, 2007) and even for tropical rainforest (Engelbrecht et al., 2007). Water availability affects plant survival (Engelbrecht and Kursar, 2003; Matías et al., 2011), and therefore species distribution (Engelbrecht et al., 2007; Valladares, 2008).

Plants have developed several drought strategies to cope with these adverse drought conditions (Joffre et al., 1999; Chaves et al., 2002; Valladares, 2008). Drought-tolerant species are defined as species that are able to maintain photosynthetic activity at low levels of water availability. These are deep-rooted trees and shrubs that maintain evergreen sclerophyllous leaves during the dry summer period. In contrast, drought-avoiding species lose part of their leaves during summer, whereas geophytes and annual herbs avoid the dry season by completing their annual cycle before the start of summer drought. Coexisting species may differ therefore considerably in their tolerance and response to water limitations and in their ecophysiological traits (Ogaya and Peñuelas, 2003).

In water-stressed environments, plants face a trade-off between carbon gain and water loss (Cowan and Farquhar, 1977). When leaves close their stomata to avoid water loss due to low water availability in the soil (Zweifel et al., 2007) this also implies a decreased CO_2 uptake, and a reduced tree growth and forest productivity (Quero et al., 2006; Valladares and Sánchez-Gómez, 2006). Therefore, it would be expected that species adapted to drought have a slow inherent growth rate (but see Fernández and Reynolds, 2000).

The reasons why some species survive drought while others do not, is still not completely understood (Chaves et al., 2002;

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McDowell et al., 2008). Several root- stem- and leaf traits seem to be responsible for the different levels of tolerance to drought stress. For example, the ability to produce more roots than shoots has been positively related to seedling drought survival (Lloret et al., 1999) as it may enhance plant water uptake. Similarly, seedlings with a high root:shoot ratio (R:S) and a low leaf area performed better under drought (Leiva and Fernández-Aléz, 1998). However, in a field experiment with eight Mediterranean woody species, Matías et al. (2012) did not find a clear trait related to drought response.

Not only biomass allocation to roots, but also rooting depth is important, as water availability is higher in deeper soil layers. Woody species from drier environments with a longer dry season have deeper roots (Filella and Peñuelas, 2003; Sack et al., 2003; Markesteijn and Poorter, 2009), and higher maximum rooting depth per leaf area (Paz, 2003). Other root morphological traits can be related to drought tolerance. Root morphology can be described with the specific root length (SRL), which indicates how much root length can be build per unit of root mass (Ryser, 2006). A high SRL can be an advantage in water-limited conditions (but see Wright and Westoby, 1999), as maximizing SRL means an increased root–soil interface for the same carbon investment, and hence, a higher root absorption potential (Eissenstat, 1992).

Continued water transport during drought depends also on stem traits, such as wood density (WD). Tropical tree species with high WD are more cavitation resistant than species with low WD (Markesteijn et al., 2011), probably because they have narrower vessels and structurally better enforced stem material, that is more resistant to vessel implosion. In contrast, species with low WD have fast growth because of their low stem construction costs, and because the high assimilation rates that come along with their conductive stem tissues (Ter Steege and Hammond, 2001; Santiago et al., 2004).

Leaf traits determine water loss, and are therefore closely related to drought tolerance (Tardieu, 2005). A high specific leaf area (SLA) implies a high surface to volume ratio of leaves, and hence high water loss. Similarly, high photosynthetic rates imply high transpiration rates, and hence, lower drought tolerance (Valladares and Sánchez-Gómez, 2006).

This study focuses on Mediterranean ecosystems, because they are especially strongly limited by a long and intense drought period that coincides with the hottest period (with maximum daily temperatures up to 45 °C). Moreover, in the next decades, the Mediterranean region of the Iberian Peninsula is predicted to face a 20% decrease in precipitation, and an increase of 2–3 °C in temperature (MARM, 2009), all of which will lead to higher water stress. Plant establishment in the Mediterranean region is currently severely limited by drought (Rey and Alcántara, 2000; Quero et al., 2008; González-Rodríguez et al., 2011) and climatic predictions and field simulations suggest that this will become even more problematic in the future (Matías et al., 2012).

Here, a dry-down experiment was carried out with seedlings of 10 Mediterranean woody species where 17 key morphological and physiological traits were measured, with the main objective to test whether they are indeed important for the drought survival and distribution of species. This study focused on seedlings, as they are especially vulnerable to drought because of their limited root systems (Tyree et al., 2003; Markesteijn and Poorter, 2009). The experiment was carried out under controlled conditions, to be able to compare species under the same stress conditions, which is difficult to realize in the field. Most experimental drought studies compare plant performance under low and high water availability (e.g. Sack, 2004). The strength of this study resides in the fact that a dry-down experiment was done, thus simulating plant performance when the plants enter the dry season, which presents the major bottleneck for their survival. Another strong point is that many plant traits were included, as growth and its components (LAR and NAR), biomass allocation, and morphological and physiological leaf and root traits which allow to tease apart which traits are the strongest drivers of drought performance.

The following hypotheses were tested: (i) seedlings of 10 Mediterranean woody species differ strongly in their response to drought, (ii) specific functional traits will be good predictors of survival under drought, and different plant strategies can be distinguished based on functional traits and drought response, and (iii) drought survival and its underlying traits can explain the observed species distribution patterns in the field.

2. Material and methods

Research was carried out in a greenhouse at the University of Córdoba (37° 51′ N, 4° 48′ W; at an altitude of 100 m; Spain), between May 2009 and July 2010. During this period, temperature in the greenhouse was 18.8 ± 4.6 °C (mean of 14 months ± SD, with a maximum of 41 °C in summer period -on average-), relative humidity was 29.7 ± 21% and irradiance on a clear day (measured at 13.30 PM solar time, 29th of July of 2009) was 428 ± 128 μmol m^{-2} s^{-1}.

Ten Mediterranean woody species were selected from different families, differing in leaf habit (deciduous and evergreen), growth form (shrub and trees) and distribution along a drought gradient in the field (Table 1), thus adding ecological reality, and allowing for a stronger generalization of the results. To describe the position of species along the drought gradient, a mean Drought Distribution Index (DDI) was assigned to each species, based on the expert judgement of 5 researchers (see Table S2 Supplementary data on line for researchers information), following the criteria of Niinemets and Valladares (2006), which served to establish the different ranges. DDI varied from 1 (very intolerant); 2 (intolerant); 3 (moderately tolerant); 4 (tolerant) and 5 (very tolerant), based on the characteristics of the habitat distribution of the species (see Table 1 for species ranking).

2.1. Experimental design

The experiment consisted of two phases: a six-months growing phase (from May to October 2009), in which plants were grown under favourable growth conditions and watered three times a week to field capacity; and a nine-month dry-down phase (from October 2009 to July 2010) in which watering was withheld, and seedlings were exposed to acute drought until they died. At the same time a control group (5 seedlings per species) was periodically watered, which served as a comparison for the drought treatment.

2.2. Growing phase

One-year old seedlings were obtained from the San Jeronimo nursery (Consejería de Medio Ambiente, Junta de Andalucía, Spain). Seedlings were transplanted to 4.33 L PVC pots of 50 cm height and 10.5 cm diameter. Such deep pots allow deep root development without much root distortion. The substrate was a mixture of sand, peat and vermiculite (4.5:2:1). To avoid nutrient limitation, 10 g of slow release fertilizer (Plantacote Plus, NPK 14:9:15, Aglukon, Valencia) was added to each pot, and all pots were watered three times a week to field capacity. The first two months (May and June, 2009), pots were randomly distributed in a nursery (62 individuals per species; 620 plants in total). Once all plants had acclimated, a first harvest was carried out for 12 individuals per species (see methods below). Then, pots were moved to the greenhouse (now there were 50 individuals per species, 500 plants in total). Most

Table 1
Species and families used in the study, code for the species and some ecological characteristics of the species: leaf habit (D: deciduous; E: evergreen), growth form (T, tree; Sh, shrub), Drought Distribution Index (DDI), habitat characteristics and distribution in the Iberian Peninsula. Nomenclature and ecological characteristics are from Blanco Castro et al. (1997) and López González (2001). DDI was assigned to each species, based on the expert judgement of 5 researchers following the criteria of Niinemets and Valladares (2006), which served to establish the different ranges. DDI varied from 1 (very intolerant); 2 (intolerant); 3 (moderately tolerant); 4 (tolerant); 5 (very tolerant), based on the characteristics of the habitat distribution of the species.

Species	Species code	Family	Leaf habit	Growth form	DDI	Habitat	Distribution in the Iberian Peninsula
Celtis australis L.	Cel	Ulmaceae	D	T	2.25	Fresh calcareous and non calcareous loose soils Warm temperatures. Frequently next to running waters	East and South
Cistus ladanifer L.	Cis	Cistaceae	E	Sh	4.50	Silicea, non calcareous, degradated or nutrient poor soils. Dry areas shrublands. Not tolerant to frost or waterlogging	Only missing in the North
Fraxinus angustifolia Vahl.	Fra	Oleaceae	D	T	1.75	Next to running waters and bottom of the valleys, with fresh soil and high freatic level. Fresh and shaded forest	West Mediterranean region
Nerium oleander L.	Ner	Apocynaceae	E	Sh	4.00	Riversides and ravines. It can tolerate extreme dry conditions if it can reach freatica levels or a wet period during the year. Warm temperatures	More frequent in the South and East
Olea europaea L.[a]	Ole	Oleaceae	E	T	4.50	Calcareous soils. Tolerant to high temperatures, does not resist under-9 °C. It can resist dry periods	Southern area, east to Catalonia and center until the Tajo riverside
Pinus pinea L.	Pin	Pinaceae	E	T	4.25	Sandysoils, silicea, and calcalreous fresh and deep soils. Does not tolerate long frost. Competes with *Q. ilex* subsp. *ballota*	South, west and east area (Portugal) Completely missing in the North
Pistacia lentiscus L.	Pis	Anacardiaceae	E	Sh	4.75	Shrublands and garrigues. It does not resist frost, not very tolerant to dry conditions	Mediterranean region, missing continental zones of N and NW
Quercus ilex L.[b]	Q. il	Fagaceae	E	T	4.25	No preference for any soil type. Little tolerance to winter cold	South, Central and Western areas
Quercus suber L.	Q. su	Fagaceae	E	T	2.75	Silicea, sandy soils. Sclerophyllous Mediterranean forest. Associated with mild and humid climates. Not tolerant to frost	Majority of the Peninsula
Rhamnus alaternus L.	Rha	Rhamnaceae	E	Sh	3.75	Not in calcareous soils. Tolerates well the drought Little tolerance to winter cold	Evergreen forest of Q. il., Q. sub., *Pinus halepensis* and *Pinus nigra*

[a] var. sylvestris (Mill.).
[b] subsp. ballota.

individuals were randomly distributed in four blocks, each with 11–12 plant per species, which were watered as the rest, until the drought treatment started. Also 5 plants per species were selected, which were going to be used as the control group later on the study to measure physiological traits under favourable conditions.

In October 2009, just before the start of the drought experiment and 8 weeks after the first harvest, a second harvest was carried out. For that, after a plant height assessment, 5 individuals per species were selected at random, that showed a plant height near the mean height value, therefore the extremes (plants with low and high height) were avoided. This procedure allows for a better relative growth rate estimates (Villar et al., 2008). At harvest, the plants were taken out of the pots and the soil was gently washed away from the root system. The main root was laid out on a table and its maximum length was recorded. This measurement was used to represent the maximum rooting depth of the plant, despite pot dimension constraints. Seedlings were divided into roots, stem and leaves and their fresh mass was determined. Leaves were wrapped in wet paper and placed in sealed plastic bags, and left for 24 h in the fridge for rehydration. Leaf surface was then dried with a soft paper to remove excess water and water-saturated leaf fresh mass was determined. After that, the leaves were scanned with a flatbed scanner (HP-Scanjet ADF 6300c) and leaf area was determined with the software Image-Pro Plus 4.5 (Media Cybernetics Inc.). Leaves were dried for at least 48 h at 70 °C, and the dry mass was obtained.

Table 2
Variables studied in the ten Mediterranean woody species: groups of variables, complete name, abbreviation used and units.

	Variable	Abbreviation	Units
Drought variables	Survival time	SurvT$_{50}$	Days
	Soil water content at the second week	SWC$_{2w}$	%
	Drought distribution index	DDI	Ranking
Biomass allocation and growth	Leaf mass fraction	LMF	g g^{-1}
	Stem mass fraction	SMF	g g^{-1}
	Root mass fraction	RMF	g g^{-1}
	Root:shoot	R:S	g g^{-1}
	Relative growth rate	RGR	mg g^{-1} day^{-1}
	Leaf area ratio	LAR	m^2 Kg^{-1}
	Net assimilation rate	NAR	g m^{-2} day^{-1}
	Specific leaf area	SLA	m^2 Kg^{-1}
	Leaf carbon concentration	C	%
	Stem dry matter content	SDMC	g g^{-1}
	Stem porosity	Porosity	g water cm^{-3}
Root variables	Rooting depth	RD	cm
	Rooting depth per leaf area	RDLA	cm cm^{-2}
	Specific root length	SRL	m g^{-1}
Physiological variables	Maximum photosynthetic rate per mass	A_{mass}	μmol CO_2 g^{-1} s^{-1}
	Transpiration rate per mass	E_{mass}	mmol H_2O g^{-1} s^{-1}
	Water use efficiency	WUE	μmol CO_2 mol^{-1} H_2O

A 5 cm long stem segment was taken just above the stem base. The volume of this segment was determined with the water displacement method (Olesen, 1971), and the fresh and dry mass of the stem (at least 48 h at 70 °C) were determined. The remaining stem was also dried to obtain total stem dry mass.

Roots were cleaned from the soil and a subsample of the roots was taken. Root segments were placed on a scanner (Epson Expression 164 XL) in a transparent plastic tray filled with water. Roots were scanned using WinRHIZO/2004 (Regent Instruments Inc.) and root length was obtained. Finally, scanned roots and the remainder of the roots were oven-dried at 70 °C during at least 48 h and weighed.

Based on these measurements different functional traits were calculated (see Table 2 for abbreviations and units). With help of the software Classical Plant Growth Analysis version 1.1 (Hunt et al., 2002), and using data from the two harvests, calculations were done for the relative growth rate (RGR), net assimilation rate (NAR; biomass growth rate per unit leaf area), leaf area ratio (LAR; leaf area per unit dry plant mass), specific leaf area (SLA; leaf area per unit dry leaf mass), and root to shoot ratio (R:S). Rooting depth per leaf area (RDLA) was calculated by dividing the rooting depth by the total leaf area of the plant, and specific root length (SRL) as the root length divided by the root dry mass. Stem porosity is an indicator of the amount of water that the stem can transport or store per unit volume and was calculated as: (stem fresh mass - stem dry mass)/ stem volume. Additionally, for each plant, leaf, stem and root dry matter content (LDMC, SDMC, RDMC; dry mass per unit fresh water-saturated mass), and leaf, stem and root fractions (LMF, SMF, RMF; dry mass per unit dry plant mass) were calculated.

Two weeks after the second harvest, physiological traits were measured for about five control individuals per species. Physiological traits included light-saturated photosynthetic capacity (A_{max}), transpiration rate (E) and stomatal conductance (g_s). Measurements were taken between 9 AM and to 12 PM (solar time) using a gas-exchange analyser (CIRAS-2, PP System, Hitchin, UK) with constant CO_2 concentration (380 ppm), flow (200 cm^3 min^{-1}), and leaf temperature (25 °C) in the leaf cuvette. A LED lamp provided photosynthetically active radiation of 1000 μmol m^{-2} s^{-1}. Middle-aged fully expanded leaves were selected for these measurements. In the case that leaves were not large enough to cover the whole area of the chamber, a digital picture was taken and the actual leaf area was analyzed later using Image-Pro Plus 4.5 (Media Cybernetics Inc.) to correct the gas exchange variables. Water-use efficiency (WUE) was calculated as A_{max}/g_s. Afterwards, leaves were removed, and their leaf area and leaf fresh, saturated and dry mass were determined (as described above). Leaves were manually ground in an agate mortar, and analysed for carbon (C) concentration using an elemental analyser (Eurovector EA 3000; EuroVector SpA, Milan, Italy). Mass-based assimilation (A_{mass}, μmol CO_2 g^{-1} s^{-1}) and transpiration (E_{mass}, in mmol H_2O g^{-1} s^{-1}) rates were calculated by multiplying the area-based rates with the SLA of the leaf.

2.3. Dry-down experiment

Forty seedlings per species were exposed to a dry-down experiment, and five seedlings per species were watered regularly to serve as a control group. At the start of the experiment, pots of drought-exposed plants were watered up to field capacity after which they received no additional water. Volumetric soil water content was measured weekly for four drought- and two control individuals per species using a TDR (Time Domain Reflectometry, mod 100; Spectrum Technologies, Inc., Plainfield, IL, USA) over the first 20 cm depth.

Seedling survival was initially monitored every 7–15 days for all the seedlings in the drought treatment. Individuals that had lost all their aerial structure, did not have any photosynthetically active leaf (i.e., green and flexible leaves), and exhibited loss of stem flexibility at the upper 1/3, were recorded as dead (Valladares and Sánchez-Gómez, 2006). Median drought survival time ($SurvT_{50}$) was calculated as the day when half of the individuals of each species had died. The mean survival time for each species was highly correlated with $SurvT_{50}$ ($r = 0.96$, $P < 0.001$, $n = 10$).

2.4. Data analysis

The mean values of seedlings traits (±SE) for the 10 species were calculated (Table S1, Supplementary data on line). Pearson correlations were used to evaluate how traits were related to each other, and to the drought survival time and DDI of the species. A principal component analysis (PCA) was carried out to evaluate how seedling traits were associated amongst each other. All the statistical analysis were performed using STATISTICA version 7.0 (Statsoft Inc., Tulsa, OK, USA).

3. Results

3.1. Response to drought: survival and water availability

Species differed strongly in their drought survival time ($SurvT_{50}$), ranging from 19 d for *Fraxinus angustifolia* to 192 d for *Quercus ilex* (Table S1, Supplementary data on line). Three types of survival curves could be traced (Fig. 1). The first type corresponds to species (*F. angustifolia* and *Celtis australis*) for which most of the individuals died during the first weeks of the drought. The second type corresponds to species which died more gradually (*Cistus ladanifer*, *Olea europaea*, *Quercus suber* and *Pistacia lentiscus*) and showed a nearly constant death rate. The third type consists in species that lived longer than the other two types and after some time started to die (*Q. ilex, Nerium oleander*, *Rhamnus alaternus* and *Pinus pinea*).

Soil water content in the second week of drought was positively correlated to the survival time of the species (Fig. 2a). Those species that dried the soil more rapidly during the first two weeks of drought (such as *F. angustifolia*, *C. ladanifer* and *C. australis*) had a lower survival time than others (such as *Q. ilex* or *P. pinea*), which showed a more conservative water use. Soil water content two weeks after drought was negatively correlated with the transpiration rate of the species (Fig. 2b). This suggests that a conservative strategy of water use during the first weeks of drought could enhance long-term survival.

3.2. Functional traits as indicators of drought survival time

The drought survival time was strongly and positively correlated to Rooting Depth per Leaf Area (RDLA) (Fig 3a). Species which survived longest (*Q. ilex* and *P. pinea*) had deeper root systems and smaller transpiring area. In contrast, other species which survived shortest (*F. angustifolia* and *C. australis*) had deep roots, but a considerably larger transpiring area, and therefore a low RDLA. This suggest that, species able to optimize this relation, making deep root systems to explore the lower and wetter levels of soil, and reducing the transpiring area, can resist the drought longer.

Drought survival time was also negatively correlated to the relative growth rate (RGR) and net assimilation rate (NAR) (Fig. 3b and c). Therefore, those species with fast growth and high NAR (*F. angustifolia* and *C. australis*, followed by *C. ladanifer*), were less tolerant to drought than species with a slow RGR and low NAR (*Q. ilex* and *P. pinea*).

The two first axes of the PCA analyses explained together 67% of the variation of the studied traits (Fig. 4a). The first axis, which explained 42% of variance, was positively related to drought survival time and RDLA but negatively related to RGR and NAR (Tables 3 and 4). The first axis seems to be associated with resource uptake and growth rate. On the left side (Fig. 4b) there are the species (*F. angustifolia* and *C. australis*) with a high RGR and NAR, that have productive leaves with a high SLA, high R:S and high resource uptake (i.e., a low soil water content) but a lower drought survival time. On the right side, there are the species (*Q. ilex* and *P. pinea*) with the opposite traits (a high drought survival time, RDLA, water content at the second week and leaf carbon content but slow growth and associated traits).

The second axis corresponds more to biomass allocation and root and stem variables. It was positively related to root mass fraction and stem dry matter content and negatively to LAR and SRL (Fig. 4b and Tables 3 and 4). The lower end of this axis includes

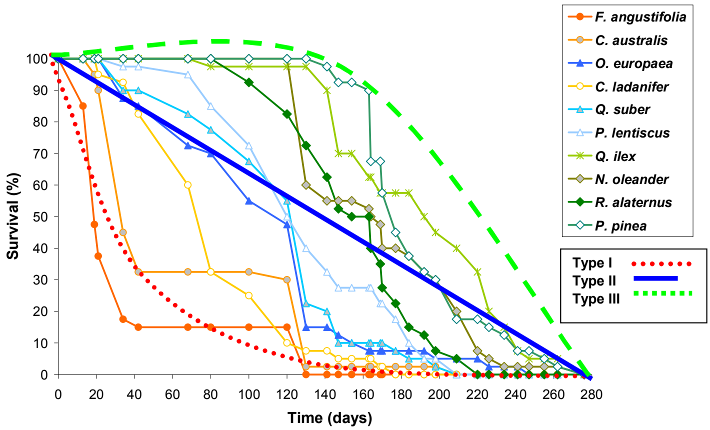

Fig. 1. Survival curves of ten Mediterranean woody species in response to drought. Percentage of survival of each species against days of a dry-down experiment. Three theoretical types of curves are drawn. Type I corresponds to species for which most of the individuals died during the first weeks of the drought. Type II corresponds to species which died more gradually and showed a nearly constant death rate. Type III are species that lived longer than the other two types and after some time started to die.

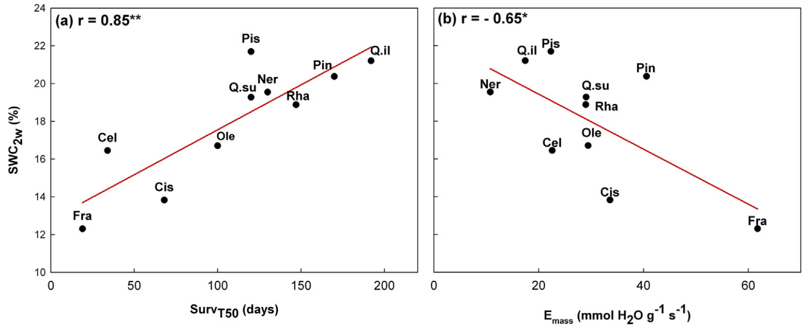

Fig. 2. (a) Drought survival time (SurvT_{50}) against soil water content at the second week of drought (SWC_{2w}), and (b) SWC_{2w} against transpiration rate (E_{mass}) in ten Mediterranean woody species. Regression lines, Pearson correlation coefficient (r), and significance levels are shown ($^*P < 0.05$; $^{**}P < 0.01$).

species (*R. alaternus*, *N. oleander* and *P. lentiscus*) with a low biomass fraction in roots but with a high SRL.

3.3. Drought and distribution patterns

The Drought Distribution Index (DDI), which indicates species' drought distribution pattern in Mediterranean forests (which ranks from 1, very intolerant, to 5, very tolerant to drought), was positive and significantly related to drought survival time (Fig. 5a). The leaf carbon concentration and leaf mass fraction were significantly and positive related to DDI (Fig. 5b, Table 3). In contrast, the root mass fraction (RMF) and the stem dry matter content (SDMC) were negatively correlated to the DDI (Table 3).

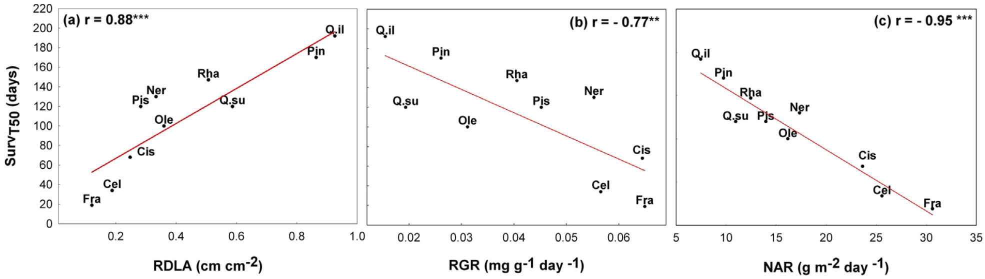

Fig. 3. Drought survival time (SurvT_{50}) against (a) rooting depth per leaf area (RDLA); (b) relative growth rate (RGR); and (c) net assimilation rate (NAR) for ten Mediterranean woody species. Regression lines, Pearson correlation coefficient (r), and significance levels are shown ($^{**}P < 0.01$; $^{***}P < 0.001$).

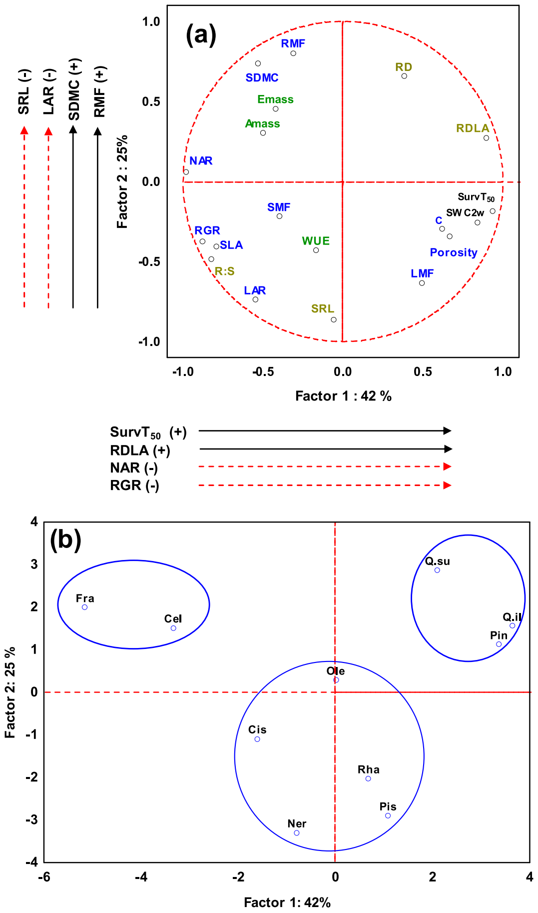

Fig. 4. Principal component analysis (PCA) for seedling traits of ten Mediterranean woody species. (a) The loadings for 19 variables on the first two PCA axes (17 seedling traits and the variable soil water content at the second week and the survival time). The arrows indicate the four variables most related to the two main factors and the direction of the relationship. (b) Scores of the species in the factor plane. The circles gather species with common strategies.

4. Discussion

Drought responses differed strongly among the ten woody species used in this study. Specific traits such as RDLA, RGR, NAR and the transpiration rate were good predictors of drought survival, and drought survival was, in turn, a good predictor of observed species distribution in the field. The species sorted out along a continuum between two recognized contrasting plant strategies as extremes; *acquisitive fast-growing species* (with soft, thin and physiologically active leaves but low drought survival) and *conservative and drought surviving species* (slow growth with C rich and sclerophyllous leaves, but high drought survival).

4.1. Drought strategies and its relationships with functional traits

The ten Mediterranean woody species differed strongly in their response to drought, in terms of their survival time and water use. The results show two extremes in the continuum of the drought response and growth. On one hand, there are some species that grow faster, have a high NAR and are heavy water users. This extreme corresponded to deciduous species (*F. angustifolia* and *C. australis*), which produce cheap leaves (i.e., high SLA with low C concentration), that are metabolically active, and have high root to shoot ratio. They have deep roots but a higher transpiring area (and therefore low RDLA). These species have the lowest drought survival time, and are found naturally distributed in relatively wet sites, as indicated by the low drought distribution index. These set of traits has been described as acquisitive traits (sensu Díaz et al., 2004). The strategy of species harbouring these acquisitive traits consists in exploiting the available resources to grow fast as long as the season is favourable. They are therefore confined to relatively resource-rich sites with high water availability.

On the other hand, the opposite extreme of the continuum belongs to evergreen species (*Q. suber*, *Q. ilex* and *P. pinea*) that are characterized by a slow RGR, with low NAR and expensive sclerophyllous leaves (high leaf C concentration). They make deep roots per transpiring area, have high soil water content at the second week, and the longest drought survival time. This strategy suggests a conservative water use as an adaptation to longer survival in drought conditions (Chunying et al., 2005). This mechanism has been observed in species with a conservative strategy (sensu Díaz et al., 2004). Several studies have demonstrated that evergreens tend to dominate sites with limiting resources, such as in dry environments (Damesin et al., 1998). *Q. ilex* and *P. pinea* have a high drought distribution index, and tend to occur on dry sites. *Quercus ilex* subsp. *ilex* is in multiple ways adapted to Mediterranean conditions; winter cold, poor soils, irregular rainfall and especially the simultaneity of the period of maximum drought with the highest temperatures of the summer heat. It also has many points of convergence with the strategies of xerophytic coniferous stands (i.e. *P. pinea*), also sclerophyllous but with needles. These two species (*Q. ilex* and *P. pinea*) show competitive interactions throughout the entire Mediterranean basin (Blanco et al., 1997; Zavala and Zea, 2004).

Table 3
Pearson correlations between traits under favourable resource conditions of seedlings of ten Mediterranean woody species, and their survival time ($SurvT_{50}$), soil water content after two weeks (SWC_{2w}), relative growth rate (RGR) and drought distribution index (DDI). Correlations in bold are significant at $0.10 > P > 0.05$ (a); $P < 0.05$ (*) or $P < 0.01$ (**). See Table 2 for abbreviations.

		$SurvT_{50}$	SWC_{2w}	RGR	DDI
Drought variables	SWC_{2w}	**0.85****			
	DDI	**0.63**a	0.52	−0.30	–
Biomass allocation and growth	LMF	0.52	0.55	−0.08	**0.82****
	SMF	−0.25	−0.42	0.24	−0.15
	RMF	−0.36	−0.30	−0.03	−**0.76****
	R:S	−**0.72***	−0.48	**0.93*****	−0.23
	RGR	−**0.77****	−**0.68***	–	−0.30
	LAR	−0.36	−0.18	**0.82****	−0.01
	NAR	−**0.95*****	−**0.88****	**0.89****	−0.58
	SLA	−0.59	−0.46	**0.85****	−0.43
	C	0.55	0.43	−0.36	**0.73***
	SDMC	−**0.72***	−0.59	0.19	−**0.74***
	Stem porosity	**0.66***	0.60	−0.48	0.52
Root variables	RD	0.22	0.20	−0.55	−0.37
	RDLA	**0.88****	**0.67***	−**0.87****	0.36
	SRL	0.12	0.08	0.38	0.41
Physiological variables	WUE	−0.02	0.15	0.11	0.02
	A_{mass}	−0.47	−0.61	0.30	−0.55
	E_{mass}	−0.48	−**0.65***	0.27	−0.47

Table 4
Eigenvector values for the variables in the two main axis of Principal Component Analysis (PCA). In bold appears the four variables more related to each axis.

		Factor 1	Factor 2
Drought variables	SurvT$_{50}$	**0.33**	−0.08
	SWC$_{2w}$	0.30	−0.12
Biomass allocation and growth	LMF	0.18	−0.29
	SMF	−0.14	−0.10
	RMF	−0.11	**0.37**
	R:S	−0.29	−0.22
	RGR	**−0.31**	−0.17
	LAR	−0.19	**−0.34**
	NAR	**−0.35**	0.03
	SLA	−0.28	−0.18
	C	0.22	−0.13
	SDMC	−0.19	**0.34**
	Stem porosity	0.24	−0.16
Root variables	RD	0.13	0.30
	RDLA	**0.32**	0.13
	SRL	−0.02	**−0.40**
Physiological variables	A_{mass}	−0.18	0.14
	E_{mass}	−0.15	0.21
	WUE	−0.06	−0.20

Overall, a trade-off between fast growth and high drought survival was found, which is in line with the growth-survival trade-off found for other limiting resources in other systems. For example, in a study with 53 rainforest species differing in shade tolerance, it was found that RGR was negatively correlated to shade survival (Poorter and Bongers, 2006), and Mediterranean woody species with high RGR (mainly deciduous oaks) showed moderate survival in response to drought (Sánchez-Gómez et al., 2006). However, Martínez-Vilalta et al. (2010) using field data from Spanish forest plots found only a weak negative relationship between RGR and survival that disappeared when phylogeny was taken into account. Similarly, Matías et al. (2012), in a field study with eight Mediterranean woody species, did not find any trade-off between growth and drought survival. The fact that these two field studies did not find a clear trade-off between survival and growth, can be partly explained by the more complex, realistic scenario under field conditions, that adds noise and may swamp more subtle relationships found under standardized conditions in controlled experiments.

Different studies have found that a deeper root system may be related to drought survival (Padilla and Pugnaire, 2007; Pinheiro et al., 2005). A deeper rooting system is advantageous as it allows plants to explore deeper and wetter soil layers for water. However, in this study, the rooting depth per se was not the best predictor of drought survival, but the ratio between rooting depth and transpiring leaf area, because it reflects a conservative strategy of water use (Paz, 2003).

Of the physiological traits evaluated in this study, only transpiration rate seemed to be important. A low stomatal conductance results in a conservative water use, high soil water levels and high drought survival. Perhaps, the explanation for the little direct relationships between physiological traits and drought performance found here is that physiological performance was measured under favourable conditions, for watered control plants. Maybe if physiological traits were measured under drought stress, a closer link to drought survival would have been found, as other authors have observed (Chaves et al., 2002; Valladares and Sanchez Gomez, 2006; Quero et al., 2011).

4.2. Species distribution pattern in relation with drought strategies

Of the 17 traits considered, the drought distribution index was most closely associated with leaf C concentration. Species from drier sites (higher DDI) had more sclerophyllous, carbon-rich leaves with high hemicellulose and lignin concentrations (Robichaux et al., 1986) and a thick cuticle. This kind of leaf is typical for Mediterranean species, which are in general adapted to dry conditions (Villar et al., 2006; Mediavilla et al., 2008).

Drought survival was indeed positively correlated with the drought distribution index, in line with our original hypothesis. Yet, drought survival explained a 42% of variation in species drought distribution in the field, suggesting that drought tolerance is not the only factor shaping the distribution of these Mediterranean species. Engelbrecht et al. (2007) found that drought sensitivity (i.e., the relative survival difference between dry and irrigated conditions) was a good predictor of species distribution along local and regional gradients of water availability. Plant water availability (as mediated through rainfall, topography, or soil characteristics) may be therefore an important factor for niche partitioning in tree species (e.g., Sterck et al., 2011). However, other factors that co-vary with water availability (such as light- and nutrient availability, or herbivore and pathogen pressure) may also shape species distribution patterns (Engelbrecht et al., 2007). Furthermore, species distributions can be the result from dispersal limitation, delayed responses to climate change, or other historical effects (Engelbrecht et al., 2007; Poorter and Markesteijn, 2008).

5. Conclusions

In this study, it was found that species were sorted along a continuum, defined between two extremes plant strategies:

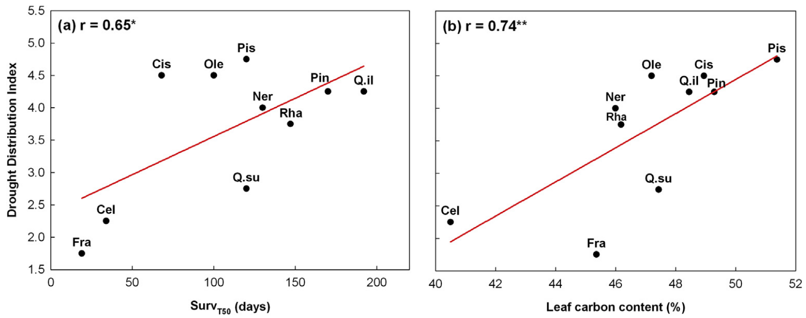

Fig. 5. Drought distribution index (DDI) against (a) drought survival time (SurvT$_{50}$) and (b) leaf carbon concentration. Regression lines, Pearson correlation coefficient (*r*), and significance levels are shown (*$P < 0.05$; **$P < 0.01$).

acquisitive fast-growing species (fast growth achieved by soft, thin and physiologically active leaves but low drought survival) and *conservative species* (slow growth with C rich and sclerophyllous leaves, but high drought survival). Along the continuum, also *intermediate species* could be found (species with a high SRL and intermediate drought survival). Species that survived drought for a long time had a high ratio of rooting depth to transpiring leaf area, and use soil water in a conservative way (low transpiration rate). Although enhanced drought survival is associated with species distribution in dry environments, it is not the only force driving species distribution. Other drivers could be dry-season growth performance, poor soils, heat stress, dispersal limitation, land-use, fire resistance and biogeographic history (Valladares, 2008).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.actao.2014.01.003.

For mean values of the variables used in this study, and some information about the researchers participating in the elaboration of Drought Distribution Index, visit the electronic supplementary information.

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Functional traits as indicators of drought performance in seedlings of ten Mediterranean woody species, by Bárbara Lopez-Iglesias$^{1, 2}$, Rafael Villar^{*,1} and Lourens Poorter^{2}

Table S1. Means ± standard error (SE, n= 5, except from LMF, SMF and RMF, n=3; and SWC_{2w}, n = 4) for plant variables used in the present study for ten Mediterranean woody species. For species codes see Table 1.

Species	$SurvT_{50}$	SWC_{2w} (%)	DDI	RGR ($mg\ g^{-1}\ day^{-1}$)	RMF	LMF	SMF	R:S	LAR ($m^2\ Kg^{-1}$)	NAR ($g\ m^2\ day^{-1}$)
Fra	19	12.31 ± 2.67	1.75 ± 0.48	64.94 ± 6.6	0.45 ± 0.04	0.18 ± 0.00	0.38 ± 0.04	0.90 ± 0.16	5.95 ± 2.22	10.92 ± 0.00
Cel	34	16.45 ± 1.29	2.25 ± 0.75	56.55 ± 7.8	0.50 ± 0.02	0.21 ± 0.01	0.29 ± 0.01	0.96 ± 0.17	6.53 ± 2.60	8.65 ± 0.00
Cis	68	13.83 ± 1.71	4.50 ± 0.50	64.50 ± 7.8	0.18 ± 0.01	0.48 ± 0.03	0.34 ± 0.02	0.81 ± 0.14	6.88 ± 2.72	9.38 ± 0.00
Ole	100	16.71 ± 2.32	4.50 ± 0.29	31.17 ± 4.6	0.20 ± 0.03	0.32 ± 0.02	0.47 ± 0.05	0.62 ± 0.22	2.70 ± 0.76	11.55 ± 0.01
Pis	120	21.70 ± 1.45	4.75 ± 0.25	45.24 ± 12.7	0.08 ± 0.00	0.65 ± 0.05	0.27 ± 0.05	0.86 ± 0.32	7.30 ± 2.74	6.20 ± 0.00
Q.su	120	19.28 ± 1.41	2.75 ± 0.25	19.37 ± 7.6	0.30 ± 0.03	0.32 ± 0.03	0.38 ± 0.02	0.36 ± 0.26	1.91 ± 0.50	10.17 ± 0.02
Ner	130	19.55 ± 0.75	4.00 ± 0.41	55.26 ± 12.9	0.24 ± 0.03	0.47 ± 0.05	0.28 ± 0.02	0.90 ± 0.25	10.32 ± 5.40	5.36 ± 0.00
Rha	147	18.88 ± 1.46	3.75 ± 0.63	40.58 ± 6.9	0.18 ± 0.05	0.51 ± 0.05	0.31 ± 0.05	0.66 ± 0.25	5.77 ± 1.85	7.04 ± 0.00
Pin	170	20.38 ± 1.26	4.25 ± 0.48	26.13 ± 4.3	0.27 ± 0.01	0.51 ± 0.05	0.22 ± 0.05	0.46 ± 0.18	3.13 ± 0.92	8.35 ± 0.00
Q.il	192	21.21 ± 1.63	4.25 ± 0.25	15.47 ± 6.6	0.38 ± 0.06	0.38 ± 0.08	0.24 ± 0.04	0.34 ± 0.49	1.81 ± 0.55	8.55 ± 0.01

Species	SLA ($m^2\ Kg^{-1}$)	SDMC	Stem porosity (g water cm^{-3})	C (%)	Rooting depth (cm)	RDLA ($cm\ cm^{-2}$)	SRL ($m\ g^{-1}$)	WUE ($\mu mol\ CO_2\ mol^{-1}\ H_2O$)	A_{mass} ($\mu mol\ CO_2\ g^{-1}\ s^{-1}$)	E_{mass} ($mmol\ H_2O\ g^{-1}\ s^{-1}$)
Fra	18.72 ± 6.8	0.50 ± 0.02	0.47 ± 0.02	45.36 ± 0.34	59.80 ± 13.5	0.12 ± 0.04	6.25 ± 2.6	76.84 ± 6.7	318.94 ± 31.5	61.84 ± 4.4
Cel	12.59 ± 4.2	0.55 ± 0.01	0.49 ± 0.01	40.50 ± 0.97	59.30 ± 5.6	0.19 ± 0.05	8.55 ± 2.7	88.51 ± 17.7	101.69 ± 17.8	22.62 ± 5.0
Cis	10.42 ± 3.9	0.40 ± 0.02	0.57 ± 0.04	48.94 ± 0.31	55.60 ± 2.0	0.25 ± 0.06	20.65 ± 8.4	54.33 ± 5.0	130.78 ± 17.3	33.65 ± 2.8
Ole	5.75 ± 1.5	0.41 ± 0.02	0.64 ± 0.02	47.20 ± 0.32	46.16 ± 10.4	0.36 ± 0.15	9.95 ± 0.8	106.80 ± 10.6	150.54 ± 16.9	29.43 ± 4.3
Pis	10.78 ± 4.5	0.35 ± 0.02	0.72 ± 0.03	51.38 ± 0.58	48.30 ± 3.1	0.28 ± 0.11	19.46 ± 9.6	104.61 ± 15.5	127.61 ± 21.4	22.29 ± 2.2
Q.su	5.24 ± 1.5	0.45 ± 0.01	0.67 ± 0.01	47.43 ± 0.08	105.40 ± 18.3	0.59 ± 0.17	2.95 ± 0.7	83.43 ± 9.1	143.23 ± 22.1	29.07 ± 3.5
Ner	18.99 ± 14.0	0.26 ± 0.02	0.58 ± 0.03	45.99 ± 1.09	54.30 ± 7.7	0.33 ± 0.08	20.42 ± 6.2	139.96 ± 9.5	82.17 ± 15.1	10.96 ± 2.0
Rha	10.86 ± 4.4	0.34 ± 0.01	0.82 ± 0.09	46.18 ± 0.69	55.10 ± 6.4	0.51 ± 0.14	32.23 ± 23.0	101.00 ± 5.5	186.43 ± 9.2	28.96 ± 2.3
Pin	4.55 ± 1.3	0.35 ± 0.03	0.77 ± 0.02	49.29 ± 0.51	68.70 ± 7.2	0.86 ± 0.12	8.30 ± 1.5	67.82 ± 4.4	135.53 ± 13.2	40.55 ± 4.3
Q.il	3.75 ± 1.1	0.38 ± 0.01	0.65 ± 0.05	48.45 ± 0.27	71.20 ± 9.1	0.93 ± 0.17	5.14 ± 1.2	101.21 ± 13.4	102.89 ± 17.7	17.43 ± 2.5

Table S2. Researchers that participated in the elaboration of Drought Distribution Index. As mentioned in section 2, they assigned an index value to each species, following the criteria of Niinemets and Valladares (2006), which served to establish the different ranges. DDI varied from 1 (very intolerant); 2 (intolerant); 3 (moderately tolerant); 4 (tolerant); 5 (very tolerant), based on the characteristics of the habitat distribution of the species.

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Capítulo 4. Drought changes the structure and elemental composition of very fine roots in seedlings of ten woody tree species. Implications for a drier climate

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REGULAR ARTICLE

Drought changes the structure and elemental composition of very fine roots in seedlings of ten woody tree species. Implications for a drier climate

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Abstract
Background and aims Water availability is often one of the most limiting factors for plants. Climate change predictions for many areas suggest an intensification of water limitation. The ability of a plant to modify its root characteristics can be an important mechanism for preventing drought stress.
Methods We studied the drought response of seedlings of 10 woody species and compared the biomass allocation, vertical root distribution across different root diameters, and the key traits of very fine roots (root diameter <0.5 mm) under two water regimes (no water limitation and severe drought).
Results Under drought conditions, the very fine roots had a higher specific root length (SRL, root length: biomass ratio), smaller root diameter and higher root tissue mass density, as well as a lower nitrogen concentration. A higher value of the mean root plasticity index was related to higher drought resistance. A quantitative literature review showed that there was a wide variation in the effect of the drought on SRL, thus there was not a clear effect of drought on SRL.
Conclusions Certain species have the necessary root traits and plasticity to survive drought. We have identified plasticity in root characteristics as a whole-plant trait which plays a significant role in separating out species into those which are vulnerable and those which are resistant to drought.

Keywords Biomass allocation · Drought · Plasticity · Vertical root distribution · Root traits · Specific root length · Survival

Introduction

Water availability is generally the most limiting factor for plants in many environments, as it affects many aspects of fitness - including growth, survival and, therefore, plant distribution (Ciais et al. 2005; Bigler et al. 2006; Boisvenue and Running 2006; Bréda et al. 2006; Engelbrecht et al. 2007; Allen et al. 2010). Soil water availability depends at a regional scale on rainfall and evapotranspiration, and at local scales on soil properties and topography (Sperry et al. 1998). In addition to the high degree of spatial variation in water availability, there is high interannual variability in annual rainfall totals. For example, in the Mediterranean region of this study (with a mean annual rainfall of 624 mm), annual rainfall in the last 93 years has varied from 254 to 1,297 mm (AEMET, Fig. S1), leading to years with strong water stress. In addition to this variable and

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erratic pattern of annual rainfall, there has been a significant decrease in rainfall with time, with a mean decline of 2 mm per year (Fig. S1). Although in many areas of the world the annual rainfall has increased, there are also many areas where it has decreased (Walther et al. 2002; Phillips et al. 2010). Even in areas where the rainfall has not changed significantly with time, the overall increase in temperature may lead to greater aridity (Solomon et al. 2007). Therefore, it is very important to understand the effects of drought on root, plant and ecosystem functioning.

Different studies have found evidence of strong impacts of drought on ecosystems, such as an increase of plant mortality (Auclair 1993; Lloret et al. 2004; Bigler et al. 2006; Allen et al. 2010) and a reduction of net primary productivity (Ciais et al. 2005; Bréda et al. 2006). Most of the research on drought stress has been focused on aboveground plant responses and less on roots, due mainly to the difficulties in observing and studying the latter (Huang and Gao 2000; Poorter et al. 2012). Roots generally represent 20–40 % of the total plant biomass (Jackson et al. 1996, 1997; Antúnez et al. 2001; Alameda and Villar 2009; Poorter et al. 2012). Generally, plants respond to drought with a decrease in shoot biomass and an increase in root biomass (Kramer and Boyer 1995; Gregory et al. 1997; Bell and Sultan 1999; Quero et al. 2006; Poorter et al. 2012), which allows the plants to minimise water loss by transpiration and increase the efficiency of the soil exploration and water acquisition, leading to a higher probability of survival (Lloret et al. 1999).

In addition to root allocation, the vertical distribution of roots throughout the soil (hereafter vertical root distribution) is an important aspect of the plant response to water stress, as deep roots can be a strategy for tackling drought (Bell and Sultan 1999; Zhu et al. 2002; Ho et al. 2005; Padilla and Pugnaire 2007; Markejstein and Poorter 2009). During drought periods, the water content in the top soil layers is depleted by soil evaporation and plant transpiration, and plants need to have extensive fine roots at a depth where water is still available (Sponchiado et al. 1989; Blum 2002; Zhu et al. 2009). Therefore, differences in rooting depth and the ability of plants to extract soil water at depth are likely to influence plant survival (Padilla and Pugnaire 2007; Markejstein and Poorter 2009).

Roots, according to their morphology and size, have different functions (Eissenstat et al. 2000). Coarse roots (root diameter >2 mm) are responsible for anchoring the plant to the soil and for carbohydrate reserves. Fine roots (0.5 to 2 mm) are involved in water and nutrient transport. Very fine roots (<0.5 mm) play a very important role in the exploration of the soil for water and nutrient absorption, mainly due to their high surface/volume ratio (Eissenstat 1992; Ostonen et al. 2007) and because they can account for around 80 % of total root length (King et al. 2002; Guo et al. 2004). Some studies have considered that very fine roots can reflect exposure to stress that may not be apparent in shoots (Vogt et al. 1993), and that they respond to induced stresses more intensively and rapidly than other types of roots (Palátová 2002; Ostonen et al. 2007).

Drought may change the structure and function of fine roots, which are described by key root traits, such as the specific root length (SRL, m g^{-1}). The SRL indicates how much root length is built per unit of root mass (Kramer and Boyer 1995; Ryser 2006). Maximisation of the SRL increases the root-soil interface and, hence, the root absorption potential (Larcher 1995; Ostonen et al. 2007). This seems to be an advantage when water is limited, since more root length is produced per unit of biomass invested (Eissenstat 1992; Ryser 1998; Wahl and Ryser 2000). Some studies revealed an increase in SRL with drought (Nicotra et al. 2002; Ebrahim 2008; Metcalfe et al. 2008), but others were not so clear about the responses of SRL to drought (Meier and Leuschner 2007; Ostonen et al. 2007). The SRL depends on the root diameter (RD, mm) and root tissue mass density (TMDr, g cm^{-3}) (Wright and Westoby 1999; Nicotra et al. 2002). The mathematical decomposition of SRL (Ostonen et al. 2007) shows that it is equal to:

$$\mathrm{SRL} = \left[1/\left(\mathrm{TMDr} \times \mathrm{RD}^2\right)\right] \times 4/\pi \qquad (1)$$

see Appendix S1.

Therefore, to understand the causes of changes in SRL, the effects of drought on both variables (RD and TMDr) have to be investigated. Drought has been found to decrease RD (Li et al. 2001; Meier and Leuschner 2007) but increase TMDr (Meier and Leuschner 2007). Therefore, the effects of drought on RD and TMDr may counteract one another, resulting in no net change in SRL, although the structure of the fine roots changes.

Structural root traits (SRL and its components) are interrelated with elemental composition. In particular, the carbon (C) and nitrogen (N) concentrations and the C/N ratio are commonly associated with root functioning (Eissenstat et al. 2000; Valenzuela-Estrada et al. 2008; Comas and Eissenstat 2009). The N concentration of fine roots has been correlated positively with root respiration and SRL (Tjoelker et al. 2005). Species with a high root C/N ratio, which expresses the ratio of tissue components related to structure (C) versus metabolism (N), have a low relative growth rate (Villar et al. 2006). Drought can decrease the N uptake capacity (Persson et al. 1995) and the N availability in the soil (Hoad et al. 2001), which may decrease the N concentration and increase the C/N ratio of roots. However, Meier and Leuschner (2007) did not find any clear effect of drought on the root C/N ratio.

An important mechanism in the acclimatisation of plants to environmental change (for example, drought) is phenotypic plasticity. Plasticity in root systems when soil resources are limiting has been considered an important aspect of plant adaptation (Grime 1994; Grime and Mackey 2002). Sensitivity to variation in soil moisture and the capacity for plastic response may be crucial for plants living in habitats that are subject to temporal drought stress (Bell and Sultan 1999). However, there are few studies on the plasticity of root traits and its relation to drought resistance.

To understand the effect of drought on structural and functional aspects of roots, we examined the changes in several key root variables under two water regimes (no water limitation and severe drought) for seedlings of 10 Mediterranean woody species which differ in their habitat preferences (Table 1, Table S1). This study was performed under controlled conditions, because variations in root characteristics can be influenced by a number of factors (soil physical properties, humidity, etc.) (Ostonen et al. 2007) which are difficult to control under heterogeneous field conditions. Moreover, we performed a quantitative review of the scant studies on woody species dealing with the effects of drought on SRL, with the objective of elucidating general trends. We pose three questions and give corresponding hypotheses:

1. How are biomass allocation, vertical root distribution and root diameter distribution with depth (coarse, fine and very fine roots) affected by drought? We expected that, under drought, the plants would increase root biomass allocation, with an increment in the proportions of fine and very fine roots in the deeper soil.
2. What are the effects of drought on key root traits? We expected that, under drought, the plants would show a higher SRL and a higher C concentration but a lower N concentration.
3. Can the capacity to modify root characteristics be a strategy for better enduring drought? We hypothesised that a high root plasticity index plays an important role in species drought resistance and survival.

The novelty of this research lies in the study of the effect of drought on many important root variables (biomass allocation, vertical root distribution, root diameter distribution and key root traits) of 10 woody species under controlled conditions. We also want to know if root plasticity could be a mechanism of survival under drought. Moreover, we combine our experimental results with a review of the literature to see general patterns of the drought effect on SRL.

Materials and methods

Species selection and experimental design

Ten Mediterranean woody species differing in leaf habit, growth form and distribution were selected (Table 1, Table S1). These species are distributed in habitats with different water availability and therefore it is expected that they show differing responses to drought.

The experiment was carried out in a greenhouse at the University of Córdoba (Spain) (37° 51′ N, 4° 48′ W; altitude of 100 m) between May 2009 and September 2010. During the experiment, the temperature in the greenhouse was 18.8±4.6 °C with a relative humidity of 29.7±21 % (mean of 14 months±SD). During the summer, the maximum temperature was 41 °C. The irradiance was 428±128 μmol m^{-2} s^{-1} (measured at 13.30 h solar time on 29th July 2009, a clear day).

One-year-old seedlings were obtained from the San Jeronimo nursery (Consejería de Medio Ambiente, Junta de Andalucía, Spain). The seedlings were transplanted in May 2009 to 4.33-L PVC pots, 10.5 cm in diameter and 50 cm in height, filled with a substrate composed of sand, peat and vermiculite (4.5:2:1). Such deep pots allow deep root development with little distortion, compared to other pot sizes used in other experiments. To prevent nutrient limitation, 10 g of slow release fertiliser (Plantacote Plus, NPK 14:9:15, Aglukon, Valencia) were added to each pot. All pots

Table 1 Families and species used in the study, species code, functional group (D: deciduous; E: evergreen) and growth form. Nomenclature is from López González (2001)

Species	Family	Species code	Leaf habit	Growth form
Celtis australis L.	Ulmaceae	Ca	D	Tree
Cistus ladanifer L.	Cistaceae	Cl	E	Shrub
Fraxinus angustifolia Vahl.	Oleaceae	Fa	D	Tree
Nerium oleander L.	Apocynaceae	No	E	Shrub
Olea europaea L. var. sylvestris (Mill.)	Oleaceae	Oe	E	Tree
Pistacia lentiscus L.	Anacardiaceae	Pl	E	Shrub
Pinus pinea L.	Pinaceae	Pp	E	Tree
Quercus ilex L. subsp. ballota	Fagaceae	Qi	E	Tree
Quercus suber L.	Fagaceae	Qs	E	Tree
Rhamnnus alaternus L.	Rhamnaceae	Ra	E	Shrub

were watered three times a week to field capacity. All individuals were distributed randomly in the greenhouse (45 individuals per species; 450 plants, with one plant per pot).

The experiment consisted of two phases. The first was a six-month phase (from May to October 2009), in which plants were grown in the greenhouse under favourable growth conditions and watered periodically to field capacity (three times a week) to allow their establishment. In October 2009, just before the start of the second phase (drought experiment), five randomly selected seedlings per species were harvested (initial control plants; C 1) (see Fig. S2a for a scheme of the experiment).

The second phase was a nine-month dry-down phase (from October 2009 to September 2010), in which watering was withheld and the seedlings were exposed to acute drought until plant death (see Fig. S2a). Thirty-five seedlings per species were exposed to a dry-down experiment and we also subjected five individuals of each species to a watering treatment (final control plants, C 2). At the start, the pots of drought-exposed plants were watered to field capacity, after which they received no additional water.

As the experiment lasted about 1 year, these watered plants (C 2) grew exponentially, their root structure and root traits could be influenced by ontogeny and their characteristics might be very different from those of the droughted plants. Thus, comparing the total plant biomass values (Fig. S2b), we can see that the final biomass of the droughted plants (24.8±1.0 g, mean±SE) was not different (P=0.88) from that of the initial control plants (C 1; 18.7±2.5 g), but did differ (P=0.05) from that of the final control plants (C 2; 81.0±5.8 g). Ontogenic effects on biomass allocation are commonly thought of in terms of biomass, not age (Enquist and Niklas 2002). Therefore, we considered that the droughted plants should be compared with the initial control plants (C 1), as they represent the state of different root variables before they were affected by drought.

Harvest

Control (C 1), droughted and watered plants (C 2) were harvested following the same methodology. The leaves and stems were dried in the oven (70 °C for at least 48 h) to obtain the dry mass. The soil in each pot was divided into four layers (0–10, 10–20, 20–30 and 30–40 cm). Although the pot was 50 cm tall, the soil occupied 40 cm. The roots were gently washed from the soil with tap water, whilst trying to avoid the loss of roots. Later, from the five previously selected individuals, the roots of three randomly selected individuals, whose layers had been separated, were classified in three different diameter categories: coarse roots (diameter >2 mm), fine roots (between 2 and 0.5 mm) and very fine roots (<0.5 mm). This was done using a digital micrometer (Palmer Micrometer, Comecta S.A., Spain) and scissors. Most studies consider the fine roots as being those <2 mm in diameter. However, some studies (i.e., Holl 1998; Nuckolls et al. 2009) considered another, smaller category: very fine roots (diameter <0.5 mm) for their important role in the exploration of the soil for water and in nutrient absorption (Eissenstat 1992; Ostonen et al. 2007). We took, from each of the three selected individuals, a sub-sample of very fine roots from the four layers, comprising about 20 % of the very fine root biomass, to be scanned.

One of our objectives was to compare key root traits of very fine roots under the two water treatments (control and drought). Plants under the dry-down treatment were subjected to an acute drought until plant death, as we wanted to record survival time. Therefore, to compare key root traits under the two treatments, we dried and scanned the very fine roots (<0.5 mm). We checked for all 10 species that the key root traits in fresh roots were strongly correlated with those measured in dried roots. For example, the diameters of very fine roots measured in fresh and dried material were strongly correlated ($r=0.90$, $P<0.001$). The TMDr of fresh roots was also highly correlated with the TMDr of dried roots ($r=0.82$, $P<0.005$). Similarly, the SRL of fresh roots was highly correlated with the SRL of dried roots ($r=0.82$, $P<0.005$). Therefore, we considered that this methodology (calculating the key root traits using the dried roots) was the best way to compare the two treatments.

Very fine roots were placed on a scanner in a transparent plastic tray filled with water. Root length, root volume and RD were analysed and calculated using WinRHIZO Pro 2004 (Regent Instruments Inc.). Finally, the roots were oven-dried at 70 °C for at least 48 h and weighed. A sub-sample of very fine roots was ground in an agate mortar and analysed for N and C using an elemental analyser (Eurovector EA 3000, Milan, Italy).

Seedling survival and relative soil water content

For droughted plants, seedling survival was monitored every 7–15 days. For individuals that had lost their leaves due to the drought, leaves were collected each week to calculate the total leaf biomass. Individuals that did not have any photosynthetically active leaves (i.e., green and flexible leaves), and exhibited loss of stem flexibility in the upper third of the stem, were recorded as being dead (Valladares and Sánchez-Gómez 2006).

We also registered the weight of each pot every 3 days, to calculate the relative soil water content (%) as: 100 × [(weight of the pot at field capacity − weight of the pot at day i) / weight of the pot at the end of the drought period]. In the calculation, the weight of the PVC container was subtracted. A mean value of the relative soil water content along the drought phase was calculated, to show the drought intensity (Fig. S3).

Plant variables and data analysis

A summary of the different variables studied, with their description and functional roles, is given in Table 2. Leaf mass fraction (LMF), stem mass fraction (SMF) and root mass fraction (RMF) were calculated, respectively, as the dry mass of leaf, stem and root divided by the total plant dry biomass. Vertical root distribution throughout the four soil layers (0–10, 10–20, 20–30 and 30–40 cm) was calculated as the root biomass at soil layer i with respect to the total root biomass (Table 2). The proportions of coarse (>2 mm), fine (between 2 and 0.5 mm) and very fine (<0.5 mm) roots were calculated as the ratio of the root biomass of the respective RD category to the total root biomass (Table 2). The RD distribution (coarse, fine and very fine) by depth was calculated as the ratio of the root biomass of each RD category to the total root biomass for each soil layer (Table 2).

For very fine roots, the SRL was calculated as root length divided by root dry mass, and the TMDr was calculated as root dry mass divided by root volume (Table 2). The root length, root volume and average RD were a direct output of WinRHIZO.

The plasticity index for the different root characteristics of each species was calculated following Valladares et al. (2006) as:

$$\text{Plasticity index} = (\text{maximum value–minimum value}) / \text{maximum value} \tag{2}$$

considering the values of the two treatments (initial control and drought). The plasticity index is always between 0 and 1. A mean root plasticity index was calculated, as the mean of all the root trait plasticity indexes.

To summarise the effect of drought on the root traits we calculated the "Drought effect", which is

$$\text{Drought effect} = \text{mean root trait } i \text{ under drought} / \text{mean root trait } i \text{ under control} \tag{3}$$

A value of drought effect >1 means that drought increased the value of this trait, and the contrary for a value <1. The drought effect and plasticity indexes reflect different aspects. Plasticity index values close to zero indicate that the root characteristic shows little plasticity in response to changes in environmental conditions, regardless of the direction of change. The drought effect indicates the effect of drought (increase

Table 2 The variables studied and their abbreviations, units, descriptions and functional roles based on the literature

Group of variables	Variables	Abrev.	Units	Description	Functional role
Biomass allocation	Root mass fraction	RMF	g root [g plant]$^{-1}$	Root biomass relative to total plant biomass	Resource allocation for root functions (uptake of water and inorganic nutrients, anchoring the plant to the soil)
	Stem mass fraction	SMF	g stem [g plant]$^{-1}$	Stem biomass relative to total plant biomass	Resource allocation for stem functions (supporting leaves and flowers, transporting water and nutrients, light acquisition)
	Leaf mass fraction	LMF	g leaf [g plant]$^{-1}$	Leaf biomass relative to total plant biomass	Resource allocation for leaf functions (physical "platform" for the processes of photosynthesis and respiration)
Vertical root distribution	Root proportion in layer *i*		g root layer *i* [g total root]$^{-1}$	Root biomass in each soil layer *i* (0–10, 10–20, 20–30 and 30–40 cm) relative to total root biomass	Trade-offs between acquisition of resources concentrated near the soil surface and those with higher amounts at depth
Root diameter distribution	Coarse root proportion		g coarse root [g total root]$^{-1}$	Root proportion with a diameter >2 mm	Anchoring the plant to the soil, carbohydrate reserve and long-distance transport
	Fine root proportion		g fine root [g total root]$^{-1}$	Root proportion with a diameter >0.5<2 mm	Nutrient and water uptake. Dynamic root fraction, this being a significant part of net primary production
	Very fine root proportion		g very fine root [g total root]$^{-1}$	Root proportion with a diameter <0.5 mm	Nutrient and water uptake. The most dynamic root fraction, this being a significant part of net primary production
Root diameter distribution by depth	Proportion of root diameter category$_x$ in layer *i*		g root category$_x$ layer *i* [g root layer *i*]$^{-1}$	Root biomass of each root category relative to total root biomass in each soil layer *i*	Vertical root allocation, considering root diameter classes. Related to anchoring, reserve and nutrient and water uptake
Traits of very fine roots	Specific root length	SRL	m g^{-1}	Ratio of root length to root biomass	Potential resource uptake of the root
	Root diameter	RD	mm	Mean root diameter	Penetration ability and hydraulic conductivity of roots
	Root tissue mass density	TMDr	g cm^{-3}	Ratio of root mass per root volume	Related to root dry matter content and the mechanical resistance of roots during soil exploration
	C/N ratio	C/N	g C / g N	Ratio of carbon to nitrogen concentration	Trade-off in structural versus metabolic root function. An increase indicates lesser root activity

or decrease) - and its magnitude - on the root characteristic.

We defined a drought resistance index (Ds), for each of the 10 species, as the percentage of individuals that were alive after 3 months of experimental drought. We chose this length of time because it roughly corresponds to the average length of the dry season in the Mediterranean region (Quero et al. 2011).

The data were analysed with a two-way ANOVA, with species and treatment (drought and control) as factors. To analyse differences between treatments, a Tukey post-hoc test was used with a *P* level of 0.05. The data were transformed (log, arcsin) if the variables did not fulfil the assumptions of the ANOVA. Pearson correlation and regression analyses were used to analyse the relationships between variables. All statistical analyses were carried out using Statistica v8 (Statsoft, Inc., Tulsa, USA).

Quantitative review of root traits of fine and very fine roots

A quantitative review of the studies published on the effect of drought on SRL was performed. The objective was to determine if there is a general pattern in the effect of drought on SRL. We searched Google Scholar and the Science Citation Index with the keywords "specific root length" and "drought", selecting only those studies with SRL data for the fine roots of woody species under field or controlled conditions. The first conclusion was that the amount of studies dealing with the effects of drought on SRL was small. Thus, our review included only 21 studies with data for fine and very fine roots; including our data, we compiled 86 paired data of SRL (high and low water supply) (Appendix S2). We analysed the relationship of SRL under low water with SRL under high water. We used all the data available, but the data are represented according to several groups: agricultural, desert, dry tropical, Mediterranean, our study, riparian, temperate and moist tropical species. We perform a t-test for dependent samples to check if there is a significant difference in SRL under both treatments.

Results

Biomass allocation, vertical root distribution and root diameter distribution

There were significant differences in the leaf, stem and root biomass among species (Fig. 1a, Table 3). Drought led a significant increase of root biomass, but there was no significant effect on leaf and stem biomass (Fig. 1a, Table 3). Considering the proportions, there was a wide variation in the root biomass fraction among species (Fig. 1b; Table 3): *Cistus ladanifer* and *Celtis australis* showed the lowest (about 20 %) and highest (about 50 %) root mass fraction, respectively. In general, droughted plants had a higher RMF than the initial control plants (C 1) (Fig. 1b; Table 3). However, although drought decreased the LMF significantly (Table 3), it did not have any effect on the SMF (Table 3). In general, the shoot/root ratio, which depends to a great extent on the RMF and LMF, decreased significantly due to drought (Table 3). The species responded to drought in a similar way - as can be seen by the low value of the interaction term Species × Drought (Table 3).

Most of the root biomass of the initial control plants (C 1) was in the first 10 cm (about 48 % of total root biomass), showing an exponential decrease with soil depth (Fig. 2). In general, most of the species showed a similar pattern (Fig. S4; Table S2). Plants under drought had a lower proportion of their roots in the surface layer, but more roots in the deeper layers (layers 2, 3 and 4) (Fig. 2b; Table 3). The responses of the species to drought in terms of vertical root distribution varied significantly, as can be seen by the interaction term Species × Drought (Table 3, Fig. S4).

The largest part of the root biomass was composed of coarse roots (around 50 %) and there was no effect of the drought on the coarse root proportion (Fig. 3a). However, with drought, the proportion of fine roots decreased whereas that of very fine roots increased (Fig. 3a), both significantly (Table 3).

Considering the partitioning in coarse, fine and very fine roots throughout the soil, most of the roots in the upper soil layer - around 80 % - were coarse ones (Fig. 3b); this percentage decreased with soil depth. The distribution of fine roots (about 40 %) was mainly in the two middle layers of soil (10–20 and 20–30 cm) (Fig. 3c). However, the very fine roots were most abundant at 30–40 cm depth, representing 60–70 % of the root biomass in that soil layer (Fig. 3d). The drought did not have a significant effect on the proportion of coarse roots (Fig. 3b; Table 3), but it did on the roots of diameter <2 mm (fine and very fine roots) (Figs. 3c and d; Table 3). In general, the proportion of fine roots showed a significant decrease with drought (Fig. 3c) whereas the proportion of very fine roots increased significantly with drought (Fig. 3d; Table 3). These changes were clearer in the two deepest soil levels. The species responded differently to drought in relation to the distribution of fine and very fine roots (the interaction term Species × Drought was significant) (Table 3).

Root traits

Similar to the other root variables, most of the variation in the root traits was due to the species factor (Table 3). For example, the species greatly differed in the SRL of very fine roots, with minimum values for *Olea europaea*

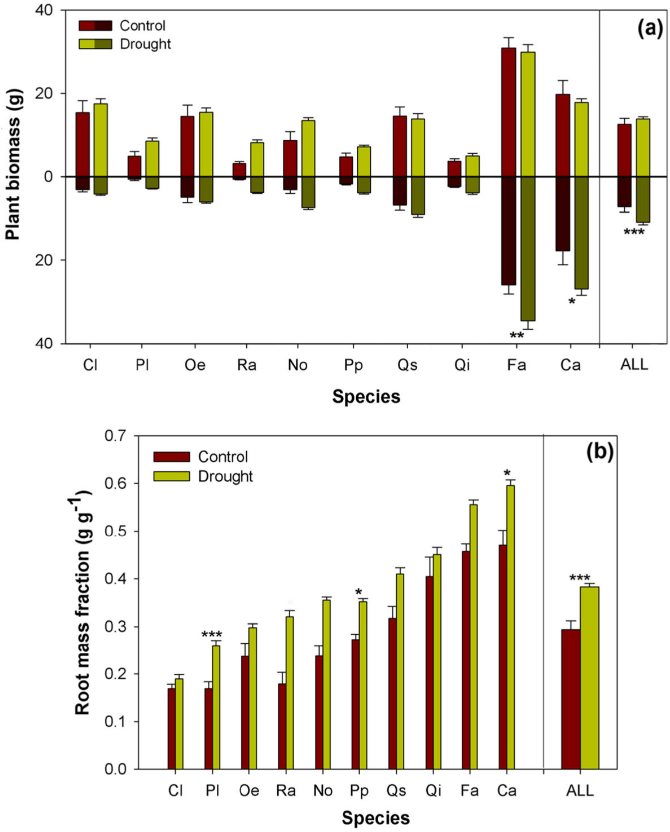


Fig. 1 Mean ± SE of **a** aboveground and belowground biomass and **b** root mass fraction (RMF), for each species and all species for the two treatments (control in *red* and drought in *yellow*). For species code see Table 1. The levels of significance (* $P<0.05$; ** $P<0.01$; *** $P<0.001$) are indicated. Species were ordered by the root mass fraction

(30±2.9 m g^{-1}) and maximum ones for *C. australis* (60 ±2.9 m g^{-1}) (Fig. 4a).

Drought also affected most of the root traits (Table 3): it significantly increased the SRL (Fig. 4a; Table 3) and TMDr (Fig. 4c; Table 3) and decreased the RD of very fine roots (Fig. 4b; Table 3). For both treatments, SRL was related negatively to TMDr, but not to RD (Fig. S5).

The N concentration of the very fine roots decreased significantly with drought (Table 3). However, drought did not have any effect on the C concentration (Table 3), so the C/N ratio increased significantly with drought (Fig. 4d; Table 3).

As a summary, we calculated the mean drought effect (see Eq. 3) for each of the root variables measured. A drought effect value >1 means that drought increased the value of this trait, and the contrary for a value <1. In general, we found increases in RMF, the proportion of very fine roots, SRL, TMDr and C/N ratio, and decreases in RD and N concentration (Fig. 5).

Root plasticity and survival

The drought resistance index (Ds) was strongly related to survival time ($r=0.94$; $P<0.001$) (data not shown). The Ds was positively correlated with the plasticity index for some root variables, such as the distribution of fine roots (in layers 2 and 3) and very fine roots (in layers 2, 3 and 4), RD and C/N ratio (Table 4). For many root variables, although the correlation was not significant, there was a trend towards being positive (Table 4). Therefore, the mean root plasticity index (the mean of the plasticity indexes of all root variables) was positively correlated with the Ds (Fig. 6), indicating that species showing a low response with regard to modification of root traits were those with a low Ds. *Pinus pinea* and *Pistacia lentiscus* had a high root plasticity index and high drought resistance, in contrast to species such as *C. australis* and *Fraxinus angustifolia,* which displayed a low root plasticity index and low drought resistance.

Table 3 Results of a two-way ANOVA in which the effect of Species, Drought and their interaction (Species × Drought) on the different variables studied are shown

Variable	Factors Species (S)	 Drought (D)	 S × D	 R^2
Biomass and proportions				
Leaf biomass	**19*****	0.3 ns	1.2 ns	**32*****
Stem biomass	**45*****	0.4 ns	0.7 ns	**63*****
Root biomass	**55*****	**2*****(+)	1.6 ns	**81*****
Root mass fraction (RMF)	**51*****	**8*****(+)	1.5 ns	**77*****
Stem mass fraction (SMF)	**35*****	0.0 ns	**4 ****	**59*****
Leaf mass fraction (LMF)	**47*****	**6*****(−)	**3*****	**69*****
Shoot/Root ratio	**43*****	**8*****(−)	**12*****	**67*****
Vertical root distribution				
Root layer 1 (Root L_1)	**27*****	**20*****(−)	**23*****	**59*****
Root layer 2 (Root L_2)	**15*****	**3*** (+)	7.7 ns	**51*****
Root layer 3 (Root L_3)	**39*****	**4*** (+)	**17****	**47*****
Root layer 4 (Root L_4)	**25*****	**16.8*****(+)	**20****	**51*****
Root diameter distribution				
Coarse root	**80*****	0.1 ns	4.5 ns	**80*****
Fine root	**64*****	**7***** (−)	**14*****	**78*****
Very fine root	**44*****	**13*****(+)	**14****	**65*****
Root diameter distribution by depth				
Coarse root				
Layer 1	**75*****	1.9 ns	4.8 ns	**75*****
Layer 2	**82*****	0.6 ns	2.3 ns	**81*****
Layer 3	**71*****	0.2 ns	4.3 ns	**69*****
Layer 4	**67*****	1.1 ns	2.6 ns	**65*****
Fine root				
Layer 1	**70*****	**3**** (−)	5.8 ns	**72*****
Layer 2	**64*****	**4**** (−)	7.1 ns	**67*****
Layer 3	**37*****	**6**** (−)	**21****	**50*****
Layer 4	**38*****	**11*****(−)	**27*****	**65*****
Very fine root				
Layer 1	**48*****	0.3 ns	12.1 ns	**48*****
Layer 2	**62*****	0.9 ns	**17*****	**75*****
Layer 3	**57*****	**5***** (+)	**20*****	**77*****
Layer 4	**41*****	**7***** (+)	**29*****	**68*****
Root traits				
Specific root length (SRL)	**69*****	**11*****(+)	**4***	**80*****
Root diameter (RD)	**34*****	**23*****(−)	7.3 ns	**56*****
Root tissue mass density (TMDr)	**59*****	**5**** (+)	2.6 ns	**58*****
Carbon concentration (C)	**29*****	2.7 ns	**16***	**35*****
Nitrogen concentration (N)	**31*****	**14*****(−)	**17*****	**52*****
Carbon/Nitrogen (C/N)	**33*****	**10*****(+)	**12***	**43*****

The proportion of the explained variance (SS_x/SS_{total}) and the level of significance (n.s., not significant; * $P<0.05$; ** $P<0.01$; *** $P<0.001$) for each factor and the interactions are indicated. R^2 is the percentage of total variance explained by the model. (+) or (−) mean that the drought has a positive or negative effect, respectively. Coarse: root diameter >2 mm, fine: 0.5 to 2 mm and very fine: <0.5 mm. Layer 1: 0–10 cm; Layer 2: 10–20 cm; Layer 3: 20–30 cm; Layer 4: 30–40 cm. Significant values are written in bold font

Quantitative review of root traits of fine and very fine roots

The quantitative review of the effect of drought on SRL showed that there was high variability in the response to drought, with some studies showing a higher SRL under drought (values above the line 1:1; drought effect >1) and others showing the opposite (values below the line 1:1; drought effect <1) (Fig. 7). The comparison of the SRL low water against SLR high water (using the t-test for dependent samples) did not show any significant difference ($P=0.72$, see graph insert in Fig. 7).

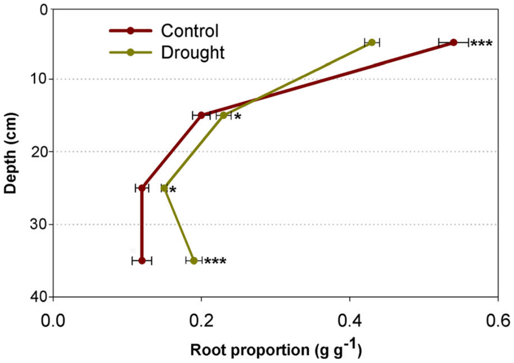


Fig. 2 Mean ± SE of vertical root distribution for the two treatments (control in *red* and drought in *yellow*). Layer 1: 0–10 cm, Layer 2: 10–20 cm, Layer 3: 20–30 cm and Layer 4: 30–40 cm. The levels of significance (* $P<0.05$ and *** $P<0.001$) are indicated

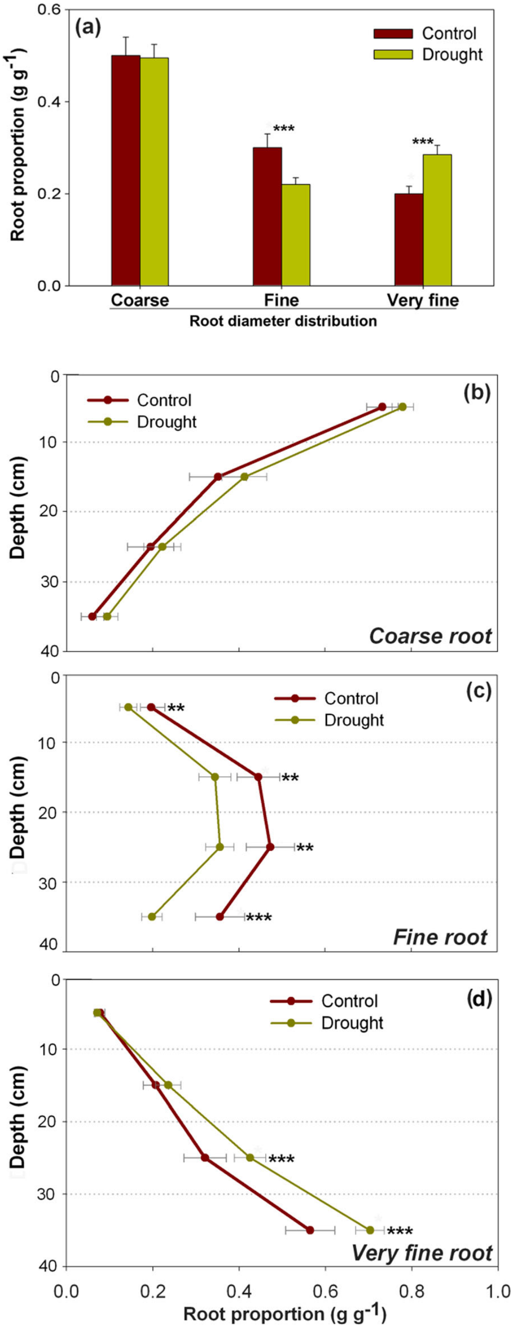


Fig. 3 Mean ± SE of **a** the proportion of root diameter distribution: coarse (root diameter>2 mm), fine (0.5 to 2 mm) and very fine (<0.5 mm) for the two treatments (control in *red* and drought in *yellow*), and root diameter distribution by depth for **b** coarse, **c** fine and **d** very fine roots. Layer 1: 0–10 cm, Layer 2: 10–20 cm, Layer 3: 20–30 cm and Layer 4: 30–40 cm. The levels of significance (** $P<0.01$; *** $P<0.001$) are indicated

Discussion

For seedlings of 10 woody species, drought increased the biomass partitioning into the roots, mainly at deeper soil levels and especially for very fine roots. Interestingly, we found that key root traits of very fine roots changed with drought, there being a higher SRL, a lower RD and a higher TMDr, but a lower N concentration. We found that species with a higher root plasticity index were more drought-resistant, which stresses the importance of root plasticity in plant performance. A quantitative review of the effect of drought on SRL showed that there is wide variation, which determines a non clear effect of drought on SRL. We discuss these results in more detail below.

Biomass allocation, vertical root distribution and root diameter distribution

Plants respond to drought by modifying their biomass allocation. Under low water availability, the proportion of root tends to increase (Bell and Sultan 1999; Poorter et al. 2012), related to the translocation of carbohydrates to the roots (Hoogenboom et al. 1987). However, the response seems to depend on the drought intensity. Several studies showed that plants subjected to moderate water stress often exhibit a small increase in RMF (Padilla et al. 2009; Poorter et al. 2012), but when plants are subjected to severe drought a strong increase in RMF is found (Poorter et al. 2012), which agrees with our results (Fig. 1b). Also, in field studies including grass, shrubland, forest and woodland ecosystems, it has been found that there is an increase in RMF with a decrease in precipitation (Mokany et al. 2006).

Most of the roots were distributed in the first 10 cm of substrate (40–50 % of total root biomass). Field studies in a Mediterranean scrub ecosystem (Martínez et al. 1998) gave similar values (51 % of root biomass in the upper 12.5 cm). This is because most of the nutrients are in the first few centimetres of soil (Ho et al. 2005). However, the distribution of root biomass throughout the soil differed between control and droughted plants. With the onset of drought, water availability in the upper

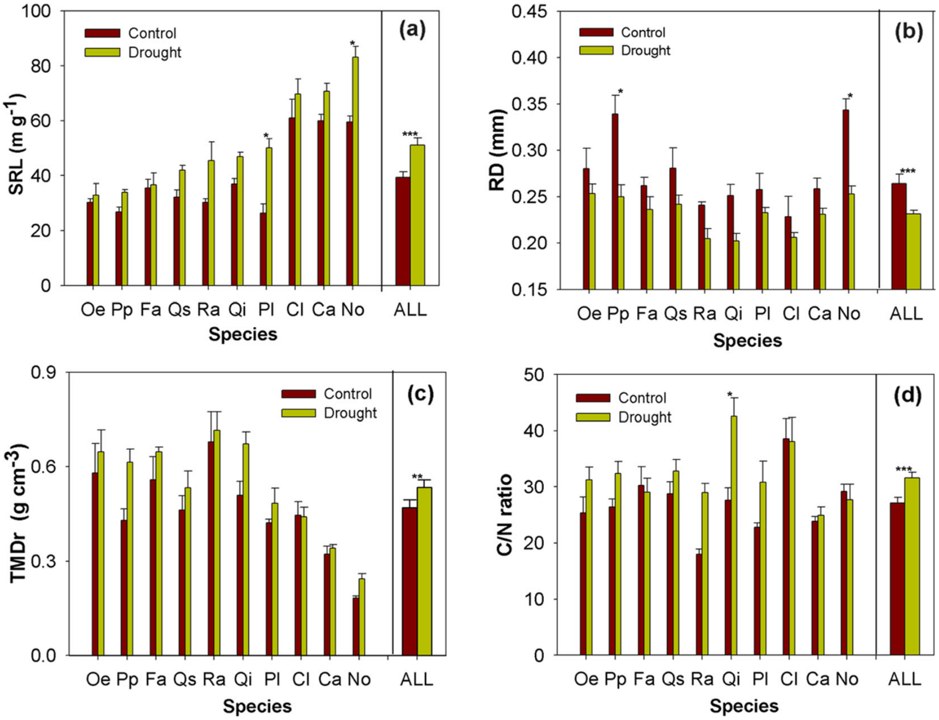


Fig. 4 Mean ± SE of key root traits for very fine roots (root diameter<0.5 mm), for each species and all species for the two treatments (control in *red* and drought in *yellow*): **a** specific root length (SRL), **b** root diameter (RD), **c** root tissue mass density (TMDr) and **d** C/N ratio. The levels of significance (* $P<0.05$; ** $P<0.01$; *** $P<0.001$) are indicated. Species were ordered by the specific root length

Fig. 5 Summary of the effect of drought on several root variables. The drought effect is calculated as the ratio of the mean value of a root trait under drought to its mean value under control conditions. The levels of significance (**$P<0.01$; *** $P<0.001$) are indicated

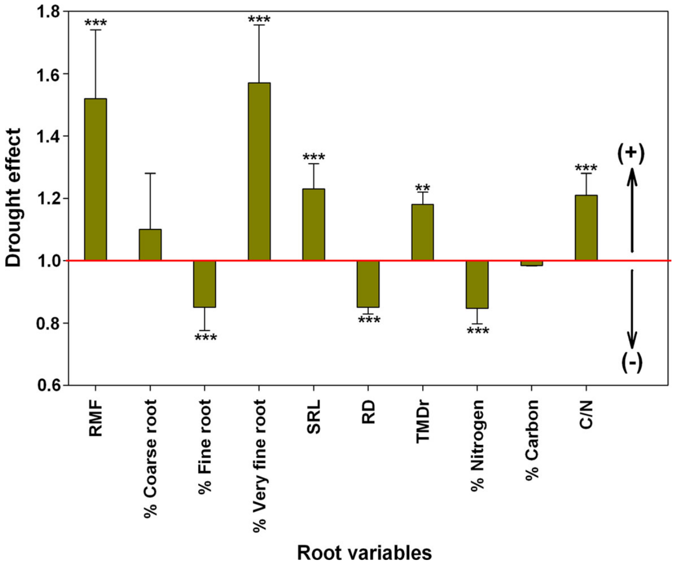


Table 4 Correlation analyses between drought resistance index and plasticity index for biomass allocation, root diameter distribution by depth and root traits

Variable	r
Biomass allocation	
RMF	0.49 ns
Root diameter distribution by depth	
Coarse root	
Layer 1	0.30 ns
Layer 2	0.40 ns
Layer 3	0.30 ns
Layer 4	−0.49 ns
Fine root	
Layer 1	0.54 ns
Layer 2	0.63*
Layer 3	0.64*
Layer 4	0.37 ns
Very fine root	
Layer 1	0.06 ns
Layer 2	0.67*
Layer 3	0.69*
Layer 4	0.72*
Root traits	
SRL	0.47 ns
RD	0.74*
TMDr	0.63a
Carbon	0.23 ns
Nitrogen	0.58a
C/N	0.67*
Mean root plasticity index	0.86**

Mean plasticity index refers to the mean of all plasticity indexes of root characteristics. Pearson product–moment correlation coefficient (r) and significance (*P*) are given: Not significant (ns) $P>0.1$; a $P<0.10$, * $P<0.05$; ** $P<0.01$. Layer 1: 0–10 cm; Layer 2: 10–20 cm; Layer 3: 20–30 cm; Layer 4: 30–40 cm

soil layer decreases: this may have caused the change in vertical root distribution to deeper levels (Fig. 2), favouring water acquisition (White and Castillo 1989; Ho et al. 2005). Bell and Sultan (1999) found greater root development in the lower layers of dry soil, compared with moist soil. Extensive, deep roots are essential for survival under water supply constraints or if the water is available in deep soil layers (Sponchiado et al. 1989; Blum 2002; Padilla and Pugnaire 2007). Deep rooting and vertical root distribution are drought adaptive traits (Passioura 1983; Taiz and Zeiger 2006; Padilla and Pugnaire 2007).

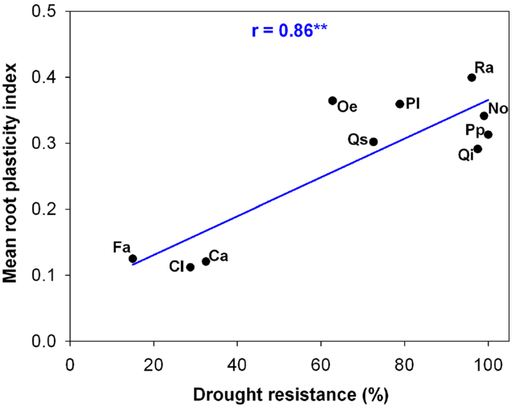


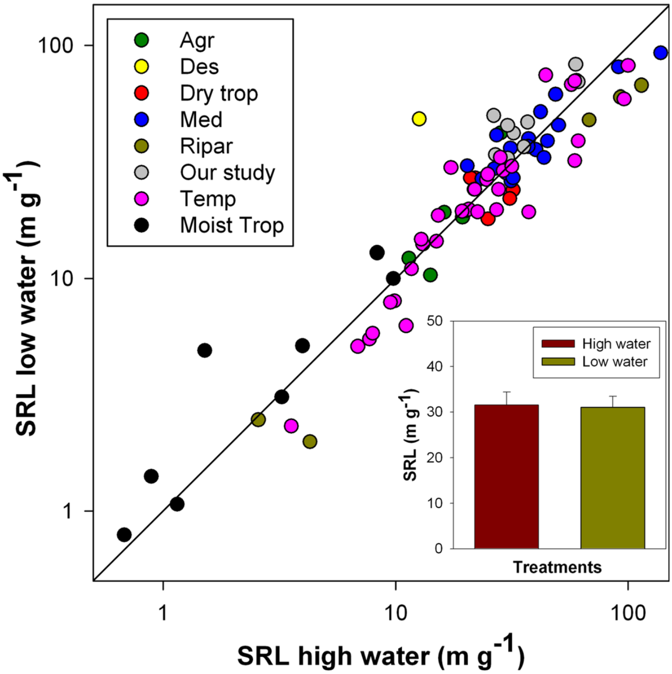
Fig. 6 Relationship between the drought survival index and the mean root plasticity index calculated using all root variables. For species code see Table 1. Pearson correlation coefficients and significance levels are given: (** $P<0.01$). We defined the drought resistance index (Ds) as the percentage of individuals that were alive after 3 months of experimental drought

As in many studies, there are potential limitations in our experiment. First, we use pots and therefore the seedlings' roots were constrained from growing horizontally. However, as all species were under the same conditions we feel that this does not change the main results. On the other hand, we studied the roots of seedlings, thus a generalization of the outcome for roots of mature trees in the field can not be made.

With regard to root morphology, we used a classification based on the RD. Some studies classified roots in different diametrical categories in order to compare species (Finér et al. 1997; Joslin et al. 2006; Pregitzer et al. 2008), but other studies, based on root order branching (Meier and Leuschner 2007; Valenzuela-Estrada et al. 2008), suggested that roots of similar diameter may differ in their elemental composition, structure and function. However, the diameter categories established in our study (coarse, fine and very fine) differ enough to affirm that different diameter categories perform different functions. Also, the use of these categories facilitates comparisons among different species.

The most dynamic RD category are the fine roots (Ostonen et al. 2007) (RD <2 mm, thus including very fine roots), since they can change faster upon environmental changes. Interestingly, drought increased the proportion of very fine roots in the lower soil layers (Fig. 3d), which may have contributed to greater drought resistance. This increase may have been due to a change in root morphology (RD reduction) or to the

Fig. 7 Relationship between SRL under low water supply and SRL under high water supply based in the literature review. The *line* represents the 1:1 line (values above the *line* indicate that drought increase SRL, values below the *line* indicate the contrary). Different groups of species are plotted with different symbols. Agr: agricultural species, Des: desert, Dry Trop: dry tropical; Med: Mediterranean; our study; Ripar: riparian; Temp: temperate; Moist Trop: moist tropical. The graph inside shows the mean (±SE) values of SRL under high and low water supply (non significant difference was found; *P*=0.72). See Appendix S2 for the references of the studies



proliferation of new, fine roots in response to soil drying. However, not all the species responded with the same intensity, as will be discussed in the root plasticity section.

Root traits

There were clear differences in key root traits of the very fine roots between the two water treatments (Figs. 4 and 5). We found that SRL and TMDr increased under water stress whereas RD decreased, which suggests that water stress increased the proliferation of very fine roots. Some studies suggested that maximisation of SRL could be particularly advantageous when water and nutrients are limited (Eissenstat 1992; Wahl and Ryser 2000; Metcalfe et al. 2008). A few studies have shown that SRL is lower under drought (Baburai 2006; Ebrahim 2008), but in the review of Ostonen et al. (2007) no clear effect of drought on SRL was found. Although our quantitative review is limited to the scant amount of studies that addressed root plasticity in woody species as a response to low water availability, the published results reveal great variability in the response to drought (Fig. 7). The great variability in the response may be due to the fact that SRL depends on both TMDr and RD (Wright and Westoby 1999; Nicotra et al. 2002; Ostonen et al. 2007; Eq. 1), since drought can affect these two variables in different ways (Ostonen et al. 2007). In fact, we found that drought had opposing effects on RD (a decrease of around 20 %) and TMDr (an increase of around 20 %) (Fig. 5), so we could expect a null effect of drought on SRL. However, this was not the case, as SRL increased with drought. This may be due to the fact that, for a similar change in TMDr and RD, RD had a higher impact on SRL than did TMDr (as RD is squared in Eq. 1).

Although thinner roots may be more vulnerable to drought (Fitter 1987), some studies have found a decrease in fine root diameter with low water availability (Fitter 1985; Baburai 2006; Cortina et al. 2008). The advantage of a smaller RD under drought may be due to the higher resistance to embolism of roots with smaller xylem vessel diameter (Alameda and Villar 2012), which is related to RD (Fitter 1987). With respect to TMDr, most studies suggest an increase in TMDr with drought (Wahl and Ryser 2000; Trubat et al. 2006; Meier and Leuschner 2007), but others do not (Cortina et al. 2008). This increase in TMDr with drought may be due to narrower vessels and higher lignification and suberisation (Steudle 2000). Also, Trubat et al. (2006) suggested that the increase in TMDr with water deficiency may be due to it coinciding with nutrient deficiency, which has a positive effect on TMDr.

We found that SRL depended on TMDr but not on RD (Fig. S5), perhaps due to the higher variation of TMDr (coefficient of variation around 30 %) compared to that of RD (around 10 %). The results of other studies have found diverse relationships, SRL depending on either RD or TMDr, or both (Comas and Eissenstat 2009). Interestingly, although in our study the variation in SRL depended on TMDr, the higher SRL under drought was caused by smaller RD.

As expected, the N concentration of the very fine roots decreased significantly with drought (Table 3). This may have been caused by lower N availability in the soil or by lower nutrient uptake, because water deficit restricts the formation of mycorrhizas - which are highly susceptible to water stress (Lehto 1992; Persson et al. 1995). However, our results do not show any significant differences in the C concentration of the very fine roots between the two treatments (Table 3). Many experiments have shown that the C concentration in roots tends to increase under drought (Hamblin et al. 1990; Gregory et al. 1997), but we did not find any clear change. Anyway, the C/N ratio, which expresses the ratio of structure versus metabolism and has been related to low growth potential (Villar et al. 2006), increased with drought (Fig. 4d) - suggesting lesser activity of the very fine roots.

Root plasticity and drought resistance

Drought resistance may be enhanced by improving the ability of the roots to extract water from the soil (Wright et al. 1994), which can be determined by changes in SRL and related traits. We found changes in root traits (SRL, diameter, tissue mass density, etc.) due to the drought treatment, which indicates an adaptive plastic adjustment (Sultan 1995). The plasticity of root characteristics can be considered a strategy, a response or both; therefore, determining whether plasticity is a mechanism of adaptation to spatially and temporarily variable environments is difficult (Bradshaw 1965; Grime et al. 1986; Sultan 2000). Some species (*P. lentiscus* and *P. pinea*) possess a high plasticity index for several root characteristics, in contrast to others (*C. australis* and *F. angustifolia*). Interestingly, the two latter species are distributed in habitats with high water availability (next to running water or in valley bottoms), which is more or less constant throughout the year (Table S1) - hence our interest in selecting representative species of a moisture gradient. We found that a high mean root plasticity index was positively related to the drought resistance index (Fig. 6). Meier and Leuschner (2007) suggested that high plasticity in root dynamics is important for higher drought tolerance in European beech. The ability to rapidly proliferate active roots in response to resource availability is likely to enhance plant success in the capture of soil nutrients and water (Lauenroth et al. 1987). According to Grime (1994), the ability to modify the root system in order to maintain vital functions and growth when the soil resources are limiting can be a key aspect of the individual's adaptive plasticity. All these results suggest that species differ in their ability to sense and respond to a given environmental stress and that this ability is closely related to their survival, so that species with higher plasticity may be more drought resistant and survive longer.

Implications for a drier climate

The changes in annual rainfall and the increase in temperature in the last 100 years have led to greater aridity in many areas of the world (Solomon et al. 2007). The results of this experiment and the general trends found in the studies to date allow us to predict possible changes in the belowground part of plants in those areas where lower annual rainfall is forecast. First, it seems clear that there will be an increase in root biomass allocation (Poorter et al. 2012), with the roots increasing comparatively more than the aboveground part, especially with a decrease in the proportion of biomass allocated to leaves; therefore, the aboveground biomass and production would decrease. Under low water availability, plants will produce roots at deeper soil depths, so that C will be sequestered at deeper levels. Drought will also increase the proportion of very fine roots, which will have a different structure and elemental composition. They will be thinner, with a higher tissue mass density, lower N concentration and high C/N ratio. Our study indicates that, although most species reacted in the same way, the species differed in the intensity of their response and a large and rapid response to drought would have the benefit of longer survival. Therefore, more studies on root characteristics - incorporating the effect of drought and other environmental changes - are needed (McCormack et al. 2012).

Conclusions

Seedlings of woody species respond to drought with changes in biomass allocation, vertical root distribution, RD distribution and root traits. Water stress induces an increase in root proportion and the development of very fine roots at deeper soil layers and with a higher SRL and lower N concentration than the roots of control plants. The mean root plasticity index seems relevant, as it was positively associated with the drought resistance index. This emphasises the importance of an integrative value of the plastic response of plant characteristics, to maintain a balanced acquisition of aboveground and belowground resources, and shows the necessity of considering a wide range of traits to fully understand plant responses to environmental conditions.

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Table S1. Species used in the study and habitat characteristics and distribution in the Iberian Peninsula. Nomenclature and data are from Blanco Castro et al. (1997) and López González (2001)

Species	**Species code**	**Habitat**	**Distribution in the Iberian peninsula**
Celtis australis L	Ca	Fresh calcareous and non calcareous loose soils. Warm temperatures. Frequently next to running water	East and South
Cistus ladanifer L	Cl	Silicea, non calcareous, degraded or nutrient poor soils. Dry area shrublands. Not tolerant to frost or waterlogging	Only absent in the North
Fraxinus angustifolia Vahl.	Fa	Next to running waters and valley bottoms, with fresh soil and high freatic level. Fresh and shaded forest	West Mediterranean region
Nerium oleander L	No	Riversides and ravines. It can tolerate extreme dry conditions if it can reach freatic levels or a wet period during the year. Warm temperatures	More frequent in the South and East
Olea europaea L var. sylvestris (Mill.)	Oe	Calcareous soils. Tolerant to high temperatures, doesn´t resist below -9 ºC. It can resist dry periods	Southern areas, east to Catalonia and centre until the Tajo river
Pinus pinea L	Pp	Sandy soils, silicea and calcareous fresh and deep soils. Does not tolerate long frost. Competes with *Q. ilex subsp. ballota*	South, West and East (Portugal). Completely absent in the North
Pistacia lentisucus L	Pl	Shrublands and garrigues. It does not resist frost and is not very tolerant to dry conditions.	Mediterranean region, absent in continental zones of North and NW peninsula
Quercus ilex L subsp. ballota	Qi	No preference for any soil type. Little tolerance to winter cold	Southern, central and Western areas
Quercus suber L	Qs	Silicea and sandy soils. Sclerophyllous mediterranean forest. Associated with mild and humid climates. Not tolerant to frost	Majority of the peninsula, forming forest
Rhamnnus alaternus L	Ra	Not in calcareous soils. Tolerates drought well. Little tolerance to winter cold	Evergreen forest of *Q. ilex*, *Q. suber*, *Pinus halepensis* and *Pinus nigra*

Table S2. Mean ± SD of root biomass, root mass fraction (RMF), stem mass fraction (SMF), leaf mass fraction (LMF), vertical root distribution (root allocation in Layer 1, 2, 3 and 4) and root diameter distribution for every species and treatment. Layer 1: 0-10 cm; Layer 2: 10-20 cm; Layer 3: 20-30 cm; Layer 4: 30-40 cm.

					CONTROL TREATMENT						
Species	**Root biomass (g)**	**RMF (%)**	**SMF (%)**	**LMF (%)**	**Root allocation L1 (%)**	**Root allocation L2 (%)**	**Root allocation L3 (%)**	**Root allocation L4 (%)**	**Coarse root (%)**	**Fine root (%)**	**Very fine root (%)**
Ca	17.74 ± 7.56	0.53 ± 0.05	0.28 ± 0.02	0.19 ± 0.03	0.35 ± 0.02	0.23 ± 0.03	0.21 ± 0.03	0.22 ± 0.04	0.61 ± 0.04	0.16 ± 0.06	0.23 ± 0.03
Cl	3.08 ± 1.2	0.18 ± 0.02	0.34 ± 0.04	0.48 ± 0.05	0.55 ± 0.10	0.15 ± 0.03	0.12 ± 0.03	0.19 ± 0.09	0.34 ± 0.05	0.29 ± 0.05	0.37 ± 0.10
Fa	25.95 ± 4.71	0.45 ± 0.06	0.38 ± 0.07	0.17 ± 0.01	0.43 ± 0.06	0.23 ± 0.03	0.17 ± 0.02	0.17 ± 0.03	0.76 ± 0.04	0.07 ± 0.01	0.17 ± 0.05
No	3.09 ± 1.81	0.25 ± 0.03	0.28 ± 0.02	0.47 ± 0.05	0.52 ± 0.05	0.23 ± 0.03	0.12 ± 0.02	0.14 ± 0.04	0.63 ± 0.13	0.21 ± 0.12	0.16 ± 0.03
Oe	4.88 ± 2.85	0.21 ± 0.03	0.47 ± 0.06	0.32 ± 0.04	0.62 ± 0.06	0.17 ± 0.05	0.11 ± 0.03	0.10 ± 0.07	0.34 ± 0.07	0.47 ± 0.10	0.19 ± 0.04
Pl	0.66 ± 0.57	0.08 ± 0.01	0.27 ± 0.09	0.65 ± 0.08	0.71 ± 0.06	0.16 ± 0.02	0.09 ± 0.03	0.04 ± 0.03	0.40 ± 0.02	0.35 ± 0.06	0.25 ± 0.08
Pp	1.72 ± 0.63	0.27 ± 0.02	0.22 ± 0.08	0.51 ± 0.09	0.54 ± 0.13	0.19 ± 0.08	0.13 ± 0.03	0.14 ± 0.10	0.38 ± 0.06	0.45 ± 0.02	0.17 ± 0.04
Qi	2.34 ± 0.46	0.38 ± 0.11	0.24 ± 0.06	0.38 ± 0.15	0.67 ± 0.02	0.19 ± 0.03	0.06 ± 0.02	0.09 ± 0.06	0.69 ± 0.09	0.21 ± 0.06	0.11 ± 0.08
Qs	6.82 ± 2.65	0.30 ± 0.06	0.38 ± 0.03	0.32 ± 0.05	0.48 ± 0.16	0.35 ± 0.10	0.09 ± 0.04	0.08 ± 0.02	0.75 ± 0.01	0.17 ± 0.01	0.08 ± 0.01
Ra	0.63 ± 0.27	0.18 ± 0.08	0.31 ± 0.08	0.51 ± 0.09	0.61 ± 0.07	0.18 ± 0.03	0.16 ± 0.06	0.05 ± 0.05	0.12 ± 0.11	0.64 ± 0.08	0.20 ± 0.07
All	7.16 ± 8.77	0.28 ± 0.14	0.32 ± 0.09	0.40 ± 0.16	0.54 ± 0.13	0.20 ± 0.07	0.12 ± 0.05	0.12 ± 0.08	0.50 ± 0.21	0.30 ± 0.18	0.19 ± 0.09

					DROUGHT TREATMENT						
Species	**Root biomass (g)**	**RMF (%)**	**SMF (%)**	**LMF (%)**	**Root allocation L1 (%)**	**Root allocation L2 (%)**	**Root allocation L3 (%)**	**Root allocation L4 (%)**	**Coarse root (%)**	**Fine root (%)**	**Very fine root (%)**
Ca	26.92 ± 9.51	0.61 ± 0.09	0.23 ± 0.09	0.16 ± 0.05	0.39 ± 0.03	0.16 ± 0.03	0.14 ± 0.01	0.31 ± 0.05	0.55 ± 0.10	0.16 ± 0.08	0.29 ± 0.05
Cl	4.12 ± 2.06	0.17 ± 0.02	0.38 ± 0.05	0.45 ± 0.04	0.58 ± 0.05	0.17 ± 0.02	0.14 ± 0.03	0.11 ± 0.02	0.33 ± 0.10	0.31 ± 0.03	0.36 ± 0.08
Fa	34.54 ± 12.56	0.53 ± 0.06	0.31 ± 0.02	0.16 ± 0.06	0.47 ± 0.05	0.19 ± 0.04	0.14 ± 0.02	0.20 ± 0.04	0.74 ± 0.11	0.09 ± 0.05	0.18 ± 0.08
No	7.43 ± 2.43	0.35 ± 0.03	0.33 ± 0.02	0.32 ± 0.03	0.38 ± 0.03	0.28 ± 0.03	0.16 ± 0.01	0.18 ± 0.02	0.73 ± 0.10	0.17 ± 0.09	0.10 ± 0.02
Oe	6.02 ± 1.9	0.29 ± 0.04	0.48 ± 0.03	0.23 ± 0.03	0.49 ± 0.08	0.18 ± 0.02	0.15 ± 0.02	0.17 ± 0.05	0.35 ± 0.13	0.33 ± 0.08	0.31 ± 0.11
Pl	2.77 ± 1.13	0.27 ± 0.05	0.30 ± 0.07	0.43 ± 0.03	0.35 ± 0.07	0.22 ± 0.07	0.15 ± 0.03	0.28 ± 0.12	0.30 ± 0.16	0.35 ± 0.12	0.36 ± 0.13
Pp	3.89 ± 1.66	0.36 ± 0.04	0.23 ± 0.03	0.42 ± 0.03	0.40 ± 0.06	0.24 ± 0.07	0.17 ± 0.02	0.19 ± 0.05	0.30 ± 0.05	0.24 ± 0.06	0.47 ± 0.09
Qi	3.87 ± 2.08	0.45 ± 0.09	0.20 ± 0.03	0.35 ± 0.07	0.48 ± 0.10	0.27 ± 0.06	0.11 ± 0.04	0.14 ± 0.05	0.63 ± 0.07	0.18 ± 0.05	0.19 ± 0.06
Qs	8.98 ± 3.86	0.39 ± 0.11	0.36 ± 0.10	0.25 ± 0.01	0.39 ± 0.03	0.37 ± 0.06	0.10 ± 0.02	0.15 ± 0.02	0.68 ± 0.02	0.15 ± 0.06	0.17 ± 0.04
Ra	3.79 ± 1.45	0.34 ± 0.11	0.32 ± 0.09	0.34 ± 0.14	0.43 ± 0.14	0.23 ± 0.09	0.16 ± 0.05	0.18 ± 0.07	0.32 ± 0.10	0.30 ± 0.04	0.37 ± 0.09
All	10.83 ± 12.49	0.38 ± 0.14	0.31 ± 0.10	0.31 ± 0.11	0.43 ± 0.09	0.23 ± 0.08	0.15 ± 0.03	0.19 ± 0.08	0.50 ± 0.2	0.23 ± 0.11	0.28 ± 0.13

Table S2 (continued). Mean ± SD of root diameter (RD), specific root length (SRL), root tissue mass density (TMDr), nitrogen (N), carbon (C), C/N ratio and survival for every species and treatment.

CONTROL TREATMENT						
Species	**RD (mm)**	**SRL (m g^{-1})**	**TMDr (g cm^{-3})**	**N (%)**	**C (%)**	**C/N**
Ca	0.26 ± 0.02	60.00 ± 4.70	0.32 ± 0.05	2.04 ± 0.09	48.46 ± 1.25	23.85 ± 1.66
Cl	0.23 ± 0.05	60.97 ± 15.37	0.45 ± 0.10	1.32 ± 0.29	49.24 ± 0.48	38.58 ± 7.99
Fa	0.26 ± 0.02	35.59 ± 6.83	0.56 ± 0.16	1.70 ± 0.30	50.11 ± 1.59	30.28 ± 6.64
No	0.34 ± 0.02	59.56 ± 4.31	0.18 ± 0.01	1.69 ± 0.16	48.89 ± 0.36	29.15 ± 2.56
Oe	0.28 ± 0.05	30.39 ± 2.88	0.58 ± 0.21	2.03 ± 0.48	48.90 ± 1.03	25.33 ± 6.42
Pl	0.26 ± 0.03	26.42 ± 6.59	0.42 ± 0.02	2.10 ± 0.13	47.82 ± 0.79	22.79 ± 1.50
Pp	0.34 ± 0.05	26.85 ± 3.98	0.43 ± 0.09	1.92 ± 0.25	50.09 ± 0.67	26.38 ± 3.25
Qi	0.25 ± 0.03	36.98 ± 4.56	0.51 ± 0.11	1.84 ± 0.31	49.63 ± 0.78	27.63 ± 5.36
Qs	0.28 ± 0.05	32.14 ± 5.85	0.46 ± 0.10	1.77 ± 0.30	49.90 ± 1.29	28.78 ± 4.74
Ra	0.24 ± 0.01	30.32 ± 2.93	0.68 ± 0.21	2.90 ± 0.39	51.68 ± 3.81	18.01 ± 2.03
All	0.27 ± 0.05	40.30 ± 18.23	0.46 ± 0.17	1.93 ± 0.48	49.53 ± 1.72	27.14 ± 6.85

DROUGHT TREATMENT						
Species	**RD (mm)**	**SRL (m g^{-1})**	**TMDr (g cm^{-3})**	**Nitrogen (%)**	**Carbon (%)**	**C/N**
Ca	0.23 ± 0.02	70.71 ± 6.55	0.34 ± 0.03	1.93 ± 0.24	47.46 ± 0.98	24.96 ± 3.20
Cl	0.21 ± 0.01	69.74 ± 12.24	0.44 ± 0.07	1.30 ± 0.30	47.18 ± 1.56	38.08 ± 9.50
Fa	0.24 ± 0.03	36.79 ± 9.21	0.65 ± 0.03	1.76 ± 0.42	49.09 ± 1.63	29.04 ± 6.02
No	0.25 ± 0.02	83.08 ± 8.93	0.24 ± 0.04	1.68 ± 0.31	45.10 ± 0.76	27.68 ± 6.22
Oe	0.25 ± 0.02	32.89 ± 9.36	0.65 ± 0.16	1.59 ± 0.26	48.49 ± 0.42	31.23 ± 5.07
Pl	0.23 ± 0.01	50.13 ± 7.45	0.48 ± 0.11	1.63 ± 0.49	47.01 ± 0.83	30.79 ± 8.54
Pp	0.25 ± 0.03	33.96 ± 2.42	0.61 ± 0.09	1.57 ± 0.25	49.91 ± 0.61	32.40 ± 4.74
Qi	0.20 ± 0.02	46.93 ± 3.58	0.67 ± 0.08	1.21 ± 0.23	50.47 ± 1.04	42.53 ± 6.58
Qs	0.24 ± 0.02	42.03 ± 3.63	0.53 ± 0.12	1.66 ± 0.29	53.51 ± 7.15	32.81 ± 4.61
Ra	0.20 ± 0.02	45.48 ± 15.25	0.72 ± 0.13	1.68 ± 0.23	48.12 ± 0.84	28.98 ± 3.63
All	0.23 ± 0.03	51.17 ± 18.43	0.53 ± 0.17	1.61 ± 0.35	48.61 ± 3.13	31.58 ± 7.23

Species	Survival (days)
Ca	54.8 ± 4.4
Cl	82.4 ± 35.8
Fa	20.8 ±7.8
No	165.2 ± 39.4
Oe	90.4 ± 35.2
Pl	147.2 ± 27.6
Pp	186.2 ± 13.2
Qi	185.2 ± 44.6
Qs	94.8 ± 40.0
Ra	147.2 ± 31.2

Appendix S1. Breakdown of specific root length into its components, following Ostonen et al. (2007).

Specific root length (SRL) describes the economy of root length production in relation to the biomass investment:

$$SRL = \frac{L}{M}$$

(Equation S1)

where *L* is the root length (m) and *M* is the root dry mass (g).

Root tissue mass density (TMDr) expresses the ratio of root dry mass to root volume:

$$TMDr = \frac{M}{V}$$

(Equation S2)

where *V* is the root volume (cm^3).

From equation S2 we can solve *M*:

$$M = TMDr \times V$$

(Equation S3)

Root radius (Rr) can be expresses as:

$$Rr = \frac{RD}{2}$$

(Equation S4)

where *RD* is the *root diameter* (mm).

Root volume (V) also can be expressed as:

$$V = \pi \times Rr^2 \times L$$

(Equation S5)

Replacing *Rr* by equation S4:

$$V = \pi \times (\frac{RD}{2})^2 \times L$$

(Equation S6)

Simplifying equation S6:

$$V = (\frac{\pi}{4}) \times RD^2 \times L$$

(Equation S7)

Replacing *V* in equation S3 by equation S7:

$$M = TMDr \times (\frac{\pi}{4}) \times RD^2 \times L$$

(Equation S8)

Replacing *M* in equation S1 by equation S8

$$SRL = \frac{L}{TMDr \times (\frac{\pi}{4}) \times RD^2 \times L}$$

(Equation S9)

Simplifying equation S9:

(Equation S10)

$$SRL = \frac{1}{TMDr \times RD^2} \times (\frac{4}{\pi})$$

Appendix S2. References used for the quantitative review of the effects of drought on SRL.

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Figure S1

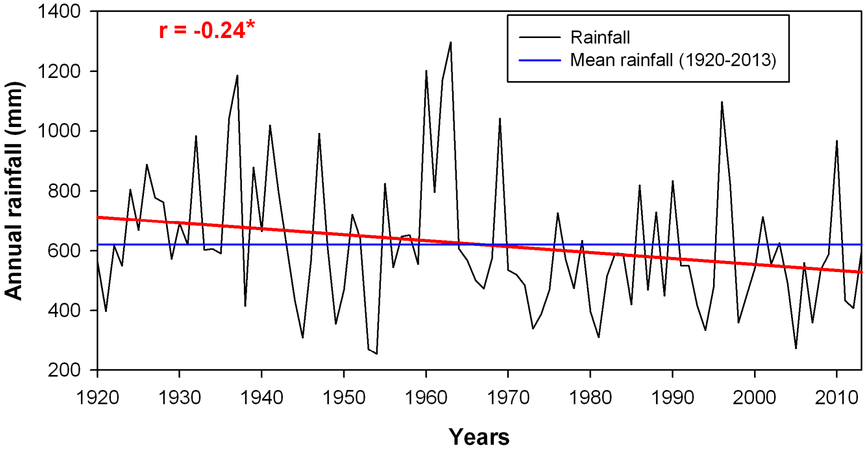

r = -0.24*
Rainfall
Mean rainfall (1920-2013)
Annual rainfall (mm)
1400
1200
1000
800
600
400
200
1920
1930
1940
1950
1960
1970
1980
1990
2000
2010
Years

Figure S2

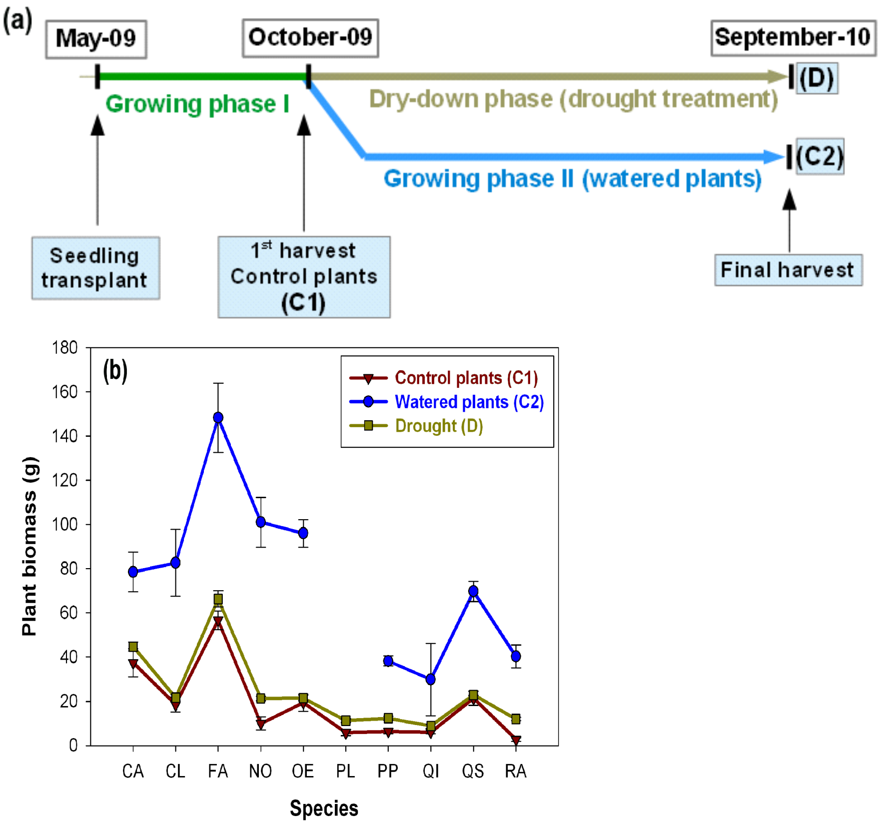

(a)
May-09
October-09
September-10
Growing phase I
Dry-down phase (drought treatment)
(D)
Growing phase II (watered plants)
(C2)
Seedling transplant
1st harvest Control plants (C1)
Final harvest
(b)
Control plants (C1)
Watered plants (C2)
Drought (D)
Plant biomass (g)
180
160
140
120
100
80
60
40
20
0
CA
CL
FA
NO
OE
PL
PP
QI
QS
RA
Species

Figure S3

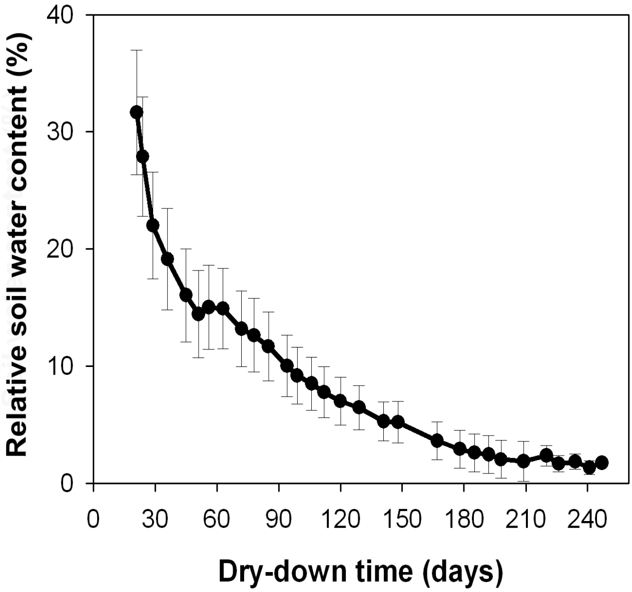

Relative soil water content (%)
40
30
20
10
0
0
30
60
90
120
150
180
210
240
Dry-down time (days)

Figure S4

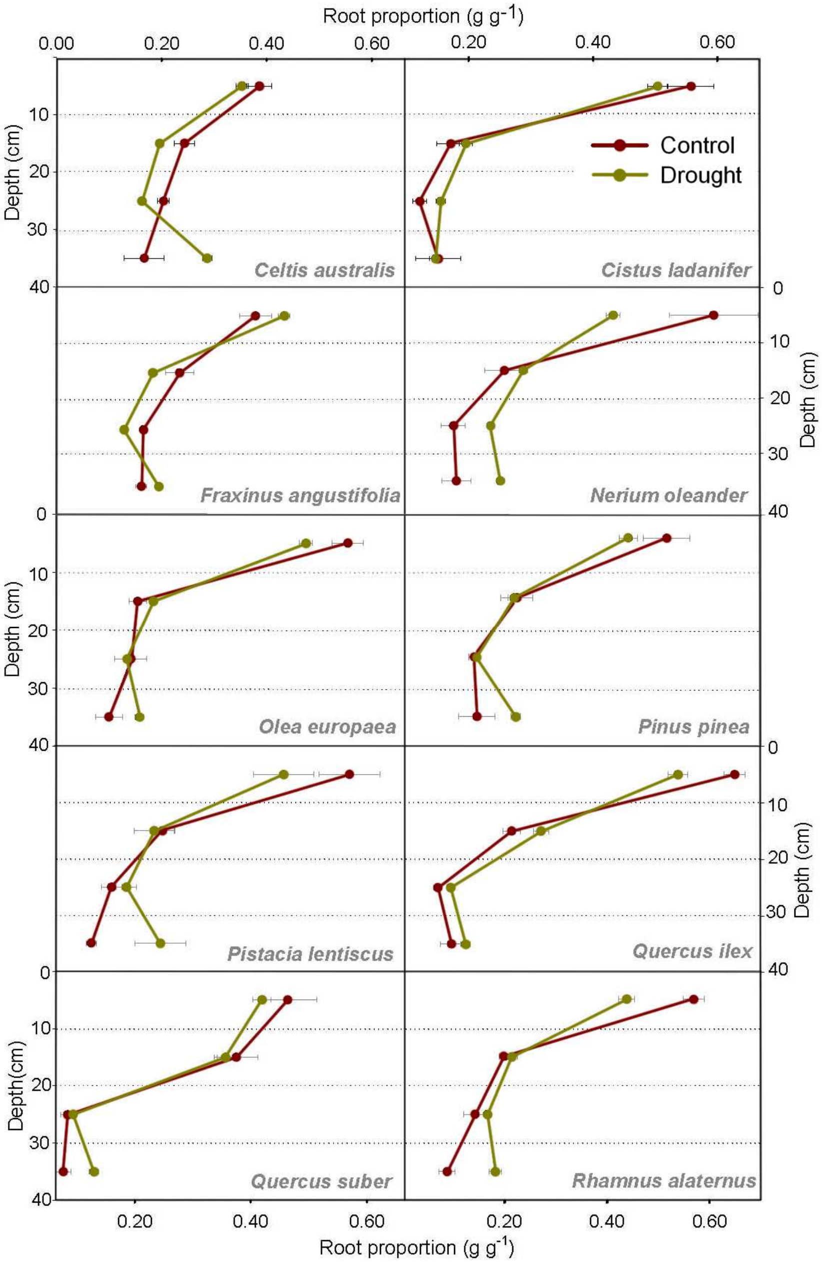
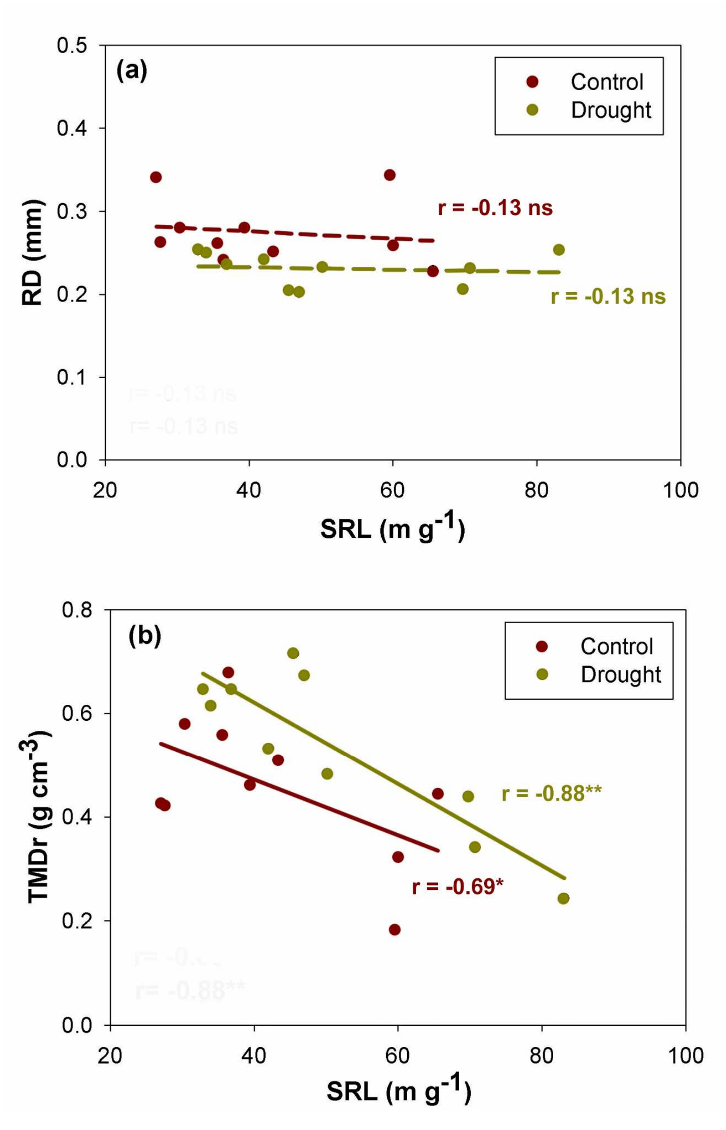

Root proportion (g g^{-1})
0.00
0.20
0.40
0.60
0.20
0.40
0.60
Depth (cm)
10
20
30
40
0
Control
Drought
Celtis australis
Cistus ladanifer
Fraxinus angustifolia
Nerium oleander
Olea europaea
Pinus pinea
Pistacia lentiscus
Quercus ilex
Quercus suber
Rhamnus alaternus
Depth(cm)
Root proportion (g g^{-1})

Figure S5


(a)
Control
Drought
r = -0.13 ns
r = -0.13 ns
RD (mm)
0.0
0.1
0.2
0.3
0.4
0.5
20
40
60
80
100
SRL (m g-1)
(b)
Control
Drought
r = -0.88**
r = -0.69*
TMDr (g cm-3)
0.0
0.2
0.4
0.6
0.8
20
40
60
80
100
SRL (m g-1)

[Fotografía: Arroyo del Molino (Córdoba)
Bárbara López]

Capítulo 5. Relationships between functional traits in green and senesced leaves and nutrient resorption in 33 woody Mediterranean species

Lopez-Iglesias and Villar. Manuscrito en preparación

REGULAR ARTICLE

Relationships between functional traits in green and senesced leaves and nutrient resorption in 33 woody Mediterranean species

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Abstract

Green leaf traits are strongly correlated to plant functioning and they also seem to have an influence on leaf litter traits, which are related to litter decomposition and the C and nutrient cycling. Before leaf fall, carbon and nutrients are reabsorbed and this is considered an important physiological mechanism that allows plants to use the same elements repeatedly. Carbon and nutrient resorption are also important because they can affect litter quality and at the end, soil fertility. There is not a clear consensuses of which are the causes of differences in the resorption efficiency between species and functional groups. For that reason, we studied structural leaf traits and C and nutrient concentrations (N, P and K) in 33 woody Mediterranean species (15 deciduous and 18 evergreens) in green and senesced leaves. The aims of this study were to know if species and functional groups (deciduous and evergreen) differ in: (i) the relationships between traits of green and senesced leaves; (ii) the resorption efficiencies of C, N, P and K; and (iii) the possible causes of the different nutrient resorption efficiencies. Our results showed that the importance of the green functional traits on the senesced leaves depends on the type of traits (structural or chemical) and on the functional groups (deciduous or evergreen) considered. The structural traits (leaf mass per area, thickness, leaf density, etc.) of green leaves had a huge effect on the structural traits of senesced leaves, irrespective of the functional group. However, the relationships of nutrients (N, P and K) concentration between green and senesced leaves were dependent on the functional group. Although for the deciduous species there was a strong relationship between nutrients of green and senesced leaves, this was not true for evergreens (which presented weak or null relationships). The resorption efficiency for all species in the study was lower for C (12.9 ± 9.1 %) and K (23.5 ± 51.6 %) than P (54.3 ± 23 %) and N (59.9 ± 13.2 %). Resorption efficiencies were similar for deciduous and evergreens, except for P, which was higher for evergreens (61.2 ± 22.8 versus 45.9 ± 20.3 %). P resorption efficiency was positively associated to the green-leaves P concentration within evergreens. Considering all species, there was not any leaf trait in green leaves that could be used as a predictor of nutrient resorption efficiency.

Key-words: deciduous; evergreen; LMA; litter; nutrient resorption efficiency; senesced-leaves

Introduction

Nutrient resorption is an important mechanism of nutrient conservation, in which nutrients are withdrawn from leaves prior to abscission and redeployed in developing tissues or stored for later use (Aerts 1996; Wright and Westoby 2003; Yasumura et al. 2005). It may occur throughout the leaf's life, particularly as leaves become progressively shaded (Ackerly and Bazzaz 1995; Hikosaka 1996), but a major pulse of resorption normally occurs shortly before leaf abscission. It is an integral part of the highly ordered process of leaf senescence and appears to occur in most species (Noodén 1988).

Through this process, about a 50% of maximum foliar N and P content can be recycled in a wide range of perennial life-forms (Aerts 1996). However, this proportion can widely vary between species. For example, <5 to 80% of leaf N, and 0 to 95% of leaf P may be resorbed (Aerts and Chapin 2000). This nutrient resorption process allows the plants to be less dependent on current nutrient uptake from the soil, which can have important ecological consequences for competition and plant fitness (van Heerwaarden et al. 2003).

Nutrient resorption is also important because it can affect litter quality and at the end, soil fertility. Both leaf-fall nutrient content and leaf-fall quantity determine tree species nutrient return and their impact on ecosystem properties (Facelli and Pickett 1991; Washburn and Arthur 2003). Leaf-fall quality largely controls leaf litter decomposition and release of nutrients into soil and thus could potentially explain species effects on soil fertility (Gallardo and Merino 1993; Cornelissen and Thompson 1997; Perez-Harguindeguy et al. 2000; Norris et al. 2012; Aponte et al. 2013; Lopez-Iglesias et al. 2014).

Resorption is typically examined as an efficiency ratio (Chapin and Kedrowski 1983; Yasumura et al. 2005) considering the nutrient concentration in green (gr) and senesced (sen) leaves as:

$$\text{RE}\ (\%) = ([\text{nutrient}]\text{gr} - [\text{nutrient}]\text{sen})/[\text{nutrient}]\text{gr} \times 100$$

Nutrient concentrations are often expressed on both mass and area bases. Mass-based concentrations do not account for changes in soluble carbon and non-target elements, which can increase or decrease during senescence. Decreases in leaf mass during senescence could underestimate mass-based resorption efficiency by 20% (Kobe, Lepczyk and Iyer 2005). That way, senesced leaves can have inflated estimates of terminal concentrations because the translocation of carbon has not been taken into account. Area-based measures do not confound target element resorption with changes in leaf mass, but leaf area shrinkage also may occur during senescence and can underestimate resorption efficiency by 10% (Aerts 1996; van Heerwaarden et al. 2003; Yasumura et al. 2005). To solve this issue, the most used method is by multiplying the ratio between green and senesced leaves nutrient concentrations by the Mass Loss Correction Factor (MLCF, i.e. the ratio between senesced and green leaves dry mass) (van Heerwaarden et al. 2003; Vergutz et al. 2012).

Within species, higher leaf nutrient status has been found to be associated with lower resorption efficiency (Lajtha 1987). In addition to leaf nutrient status, nutrient resorption has been associated in some cases to soil fertility. One hypothesis is that species adapted to nutrient-poor environments have higher resorption efficiencies (Vitousek 1982; Ralhan and Singh 1987) and lower nutrient concentrations in senesced leaves (Killingbeck 1996) as a means of nutrient conservation. However, although a link between a species' resorption efficiency and its distribution across fertility gradients is intuitively appealing, results of existing studies have been inconsistent (Aerts 1996; Kobe, Lepczyk and Iyer 2005). In some studies (Chapin and Kedrowski 1983; Lajtha 1987; Nambiar and Fife 1987) plants growing in fertile sites are more efficient at resorption, but other studies (Rahlan and Singh 1987; Chapin and Moilanen 1991) have been found the contrary (low-nutrient-adapted species have a high efficiency of nutrient resorption). In still other cases, resorption efficiency does not change with nutrient availability (Millard and Nielsen 1989; Schlesinger et al. 1989). Pugnaire and Chapin (1993) suggested that nutrient resorption is not an adaptation of plants to high or low nutrient availability, but a phenotypic response by plants to variation in nutrient status.

If soil fertility or leaf nutrient status does not follow a clear trend in their relationship with nutrient resorption (Pugnaire and Chapin 1993; Aerts, 1996; Wright and Westoby 2003), then it might be explained by other factors, as, for instance, leaf functional traits. It has been described that a major spectrum of variation runs between species with traits that favour nutrient conservation and those with traits that allow rapid short-term growth (Wright et al. 2004; Poorter et al. 2004; Lopez-Iglesias et al. 2014). Species at the nutrient-conserving end of the spectrum typically have high leaf mass per area (LMA), low leaf nutrient concentrations, and low photosynthetic capacity (Reich, Walters and Ellsworth 1997); species at the other end of the spectrum typically have the opposite characteristics. Nutrient-poor habitats tend to be dominated by nutrient-conservative species, while fertile habitats tend to be dominated by species with higher short-term productivity per leaf mass (Grime 1977; Chapin 1980; Reich 2014). Still, within any given habitat, species with a range of leaf traits may coexist (Reich et al. 1999). Wright and Westoby (2003) found that species with high LMA had also lower nutrient concentration in green leaves and lower residual nutrient concentration in senesced leaves (also called resorption proficiency, Killingbeck 1996). However, Wright and Westoby (2003) did not find any correlation of nutrient resorption with leaf traits.

We propose two reciprocally exclusive hypotheses related to nutrient resorption efficiency, functional traits and functional groups. On one hand, we could expect that deciduous species, which are metabolically more active than evergreens, might also achieve nutrient remobilization more efficiently, and therefore may also have higher nutrient resorption. Therefore, as deciduous have a lower LMA than evergreens, we would expect a negative relationship of nutrient resorption efficiency and LMA.

On the other hand, maybe evergreens, which are known to be more conservative and to have lower nutrient concentrations (Wright and Westoby 2003; Villar et al. 2006) would also be more efficient in reabsorbing nutrients, given that nutrient resorption is a nutrient conservation mechanism (Aerst 1996; Aponte et al. 2013).

In the review of Aerts (1996), with about 250 species, N resorption efficiency of evergreens (47 %) resulted significantly lower than in deciduous (54 %), whereas P resorption efficiency did not differ significantly between them (51 and 50 %, respectively). Similarly, Vergutz et al. (2012), in their global meta-analysis, found evergreens to have significantly lower resorption efficiency for C, N and K, but found no differences among functional groups regarding P. However, looking at resorption proficiency (the levels to which nutrients have been reduced in senesced leaves), Killingbeck (1996) found evergreens to be significantly more proficient at reabsorbing P than were deciduous species.

Most studies about nutrient resorption have always focused on N and P (Aerts 1996; Killingbeck 1996). Our study provides not only data on N and P, but also on C and K resorption. Potassium is also an essential nutrient, involved in photosynthesis, in the synthesis of protein and carbohydrate compounds, and also in water balance and plant cell turgidity, all of them closely related to plant growth.

In this study, green and senesced leaves were collected in 33 woody Mediterranean species (15 deciduous and 18 evergreens). Functional leaf traits and nutrient concentrations in both green and senesced leaves were measured. Resorption efficiency and proficiency was calculated for C, N, P and K. The aims of this study were to know if species and functional groups (deciduous and evergreen) differ in: (i) the relationships between green and senesced leaves traits; (ii) the resorption efficiencies of C, N, P and K; and (iii) the possible causes of the different nutrient resorption efficiencies.

Material and methods

Study site and leaves collection

Our study was conducted in Sierra Morena mountains, covering an area of 8 km of radius, in the province of Córdoba, SW Spain (37°55' N 4°50' W). The climate of this region is continental Mediterranean, with mean annual temperature of 18 °C and 600 mm of annual rainfall (Consejería de Medio Ambiente, Junta de Andalucía, Spain).

Green leaves from 33 woody plant species (15 deciduous and 18 evergreens) (Table 1) were collected, in order to cover a wide range of local species. Deciduous species were collected at autumn 2011 and evergreens at spring 2012, as these are the time of higher litter fall. Several weeks before leaf fall, branches with green leaves were collected in the field, carried to the laboratory and stored with a part of the stems submerged in water in the dark at 4 °C for 24 h. A pool of green mature leaves (5 leaves per individual, 5 individuals per species in the case of deciduous species, and 10 leaves per individual, 1 individual per species for evergreen) was selected. Leaves damaged by insects or mechanical factors were avoided.

Similarly, few weeks later, senesced leaves were collected in the field and carried out to the laboratory. Senesced leaves were collected by gently shaking the branches. Senesced leaves are those in which an abscission layer has formed in the base of the petiole, preventing further nutrient withdrawal (Norby et al. 2000). These leaves are easily identified as they have generally a different colour (often red or yellow) from live leaves and can be removed by a gentle flicking of the branch; leaves without an abscission layer are not removed by this technique (Wright and Westoby 2003). In the laboratory, senesced leaves were stored in plastic bags wrapped in humid paper in the dark at 4 °C for 24h. Similarly, leaves damaged by insects, with injuries or fungus infection were avoided.

Table 1. Family, species name, species code, growth form and plant functional type of the species in the study. Sh: shrub, S Tree: small tree; Dec: deciduous; Ever: Evergreen. Nomenclature follows Castroviejo (1986-2012).

Family	Species	Code	Growth form	Plant functional type
Anacardiaceae	*Pistacia therebintus L.*	PT	Tree	Dec
Anacardiaceae	*Pistacia lentiscus L.*	PL	Sh	Ever
Betulaceae	*Alnus glutinosa L.*	AG	Tree	Dec
Betulaceae	*Corylus avellana*	CORA	Tree	Dec
Cannabaceae	*Celtis australis L.*	CA	Tree	Dec
Caprifoliaceae	*Viburnum tinus L.*	VT	Sh	Ever
Cistaceae	*Cistus albidus L.*	CAL	Sh	Ever
Cistaceae	*Cistus crispus L.*	CC	Sh	Ever
Cistaceae	*Cistus monspeliensis L.*	CISM	Sh	Ever
Cistaceae	*Cistus ladanifer L.*	CL	Sh	Ever
Cistaceae	*Cistus populifolius L.*	CP	Sh	Ever
Cistaceae	*Cistus salviifolius L.*	CSA	Sh	Ever
Cistaceae	*Halimium atriplicifolium Lam.*	HA	Sh	Ever
Ericaceae	*Arbutus unedo L.*	AU	Sh	Ever
Fabaceae	*Ceratonia siliqua L.*	CERS	Tree	Ever
Fagaceae	*Castanea sativa Mill.*	CS	Tree	Dec
Fagaceae	*Quercus faginea Lam.*	QF	Tree	Dec
Fagaceae	*Quercus suber L.*	QS	Tree	Ever
Lamiaceae	*Phlomis purpurea L.*	PHP	Sh	Ever
Lamiaceae	*Teucrium fruticans L.*	TF	Sh	Ever
Mirtaceae	*Mirtus communis L.*	MC	Sh	Ever
Moraceae	*Ficus carica L.*	FC	Tree	Dec
Oleaceae	*Olea europaea L.*	OE	Tree	Ever
Oleaceae	*Phyllirea angustifolia L.*	PA	Sh	Ever
Rhamnaceae	*Rhamnus alaternus L.*	RA	Sh	Ever
Rosaceae	*Crataegus monogyna J.*	CM	Tree	Dec
Rosaceae	*Cydonia oblonga Mill.*	MEMB	Sh	Dec
Rosaceae	*Rosa canina*	RC	Sh	Dec
Salicaceae	*Salix pedicellata Desf.*	SP	Tree	Dec
Sapindaceae	*Acer monspessulanum L.*	AM	Tree	Dec
Simaroubaceae	*Ailanthus altissima Mill.*	AA	Sh	Dec
Thymelaeaceae	*Daphne gnidium L.*	DG	Sh	Ever
Ulmaceae	*Ulmus minor Mill.*	UM	Tree	Dec

Laboratory measurements

Similar protocols were followed for green and senesced leaves. Leaf lamina and petiole saturated fresh mass were determined separately. Then, leaves were scanned with a flatbed scanner (HP-Scanjet ADF 6300c) and leaf area was determined with the software Image-Pro Plus 4.5 (Media Cybernetics Inc., Atlanta, GA, USA). The leaf thickness (Thck) was measured with a digital micrometer (Comecta SA, Barcelona, Spain). The leaf chlorophyll content was estimated using a chlorophyll meter (CCM-200 plus, Opti-Sciences, Inc., Hudson, New Hampshire, USA) (mean value of 3 measurements), and expressed in two ways: 1)as the absolute value of chlorophyll index, and 2) as chlorophyll content per leaf thickness (Chlo/Thck). This proxy was used because the chlorophyll meter does not take into account the leaf thickness when estimating the chlorophyll content, obtaining higher values whenever the leaf is thick, and lower whenever it is thin. In addition, leaf toughness (Tough) was determined using a homemade penetrometer similar to the one described by Feeny (1976). The leaf toughness was estimated by measuring the pressure required to penetrate the leaf tissue with a cylindrical rod. We added water to a deposit above the rod until the leaf was penetrated, and converted the mass of water to penetration pressure, using the conversion formula of Gallardo and Merino (1993).

Finally, the leaves and petioles were dried separately for at least 48 h at 70 °C, and the dry mass was obtained. Leaf Mass per Area (LMA) was calculated as leaf dry-mass/leaf area and expressed in g cm^{-2}, leaf density (Dens, g cm^{-3}) was calculated following Witoski and Lamont (1991) as the ratio LMA/leaf thickness. Leaf lamina and petiole dry matter content (LDMC and DMCpet) were calculated as dry-mass/saturated-fresh mass × 100. For species with compound leaves, we considered as petiole only the fraction connecting the leaflet to the central nerve.

Chemical analyses

Chemical composition of green and senesced leaves was determined. One sample per species was homogenised using an analytical grinder (Water Cooled Analytical Grinder 2-Blade Cutter, IKA®, Germany) and was analyzed for C, N, P and K. Samples of about 2 mg were analysed for C and N concentration using an elemental analyser (Eurovector EA 3000; EuroVector SpA, Milan, Italy). Samples of about 0.25 g were digested in nitric/perchloric acid and the resulting solution analyzed for K by flame emission (Allen et al. 1976) with a flame photometer (Jenway, Bibby Scientific Limited, Staffordshire, UK); and P with the molybdenum blue colour method of Murphy and Riley (1962) and measured using an UV/VIS spectrometer (Lambda 35, Perkin Elmer Inc., USA).

Calculations

We calculated the resorption efficiency (RE), defined as the proportional withdrawal of a nutrient during senescence (van Heerwaarden et al. 2003; Yuan and Chen 2009; Vergutz et al. 2012). As mentioned in the introduction section, many proxies have been used to make these calculations. Finally, to estimate the nutrient resorption efficiency, we chose the following equation from Vergutz et al. (2012):

$$RE = [(Nut_{gr} – Nut_{sen})/Nutgr] \times MLCF \times 100$$

where Nut_{gr} and Nut_{sen} are the nutrient concentrations on a mass basis in green and senesced leaves, respectively. MLCF is the mass loss correction factor, specifically the ratio of the dry mass of senesced leaves and the dry mass of green leaves (van Heerwaarden et al. 2003; Vergutz et al. 2012) but considering similar leaf areas for both green and senesced leaves. MLCFs were calculated separately for each species. We calculated the resorption efficiency for N (N_{RE}), P (P_{RE}) and K (K_{RE}). We also calculated the resorption efficiency for carbon (C_{RE}) following the same procedure.

Statistical analysis

Mean values for each leaf trait and carbon and nutrient resorption efficiencies were calculated. To assess how green leaf traits scaled with senesced leaf traits (objective i), we used Standardised Mayor Axis regressions (SMA), using the (S)MATR: Standardised Major Axis Tests and Routines version 2.0 (Falster, Warton and Wright 2006). This software performs the SMA regression, where residuals from the regression line are estimated in both the X and Y dimensions. It calculates if the relationships are different between groups (i.e. deciduous and evergreens), the slope and elevation of the regression line, and it also tests if the slope is statistically different from 1.

To compare mean carbon and nutrient resorption efficiencies among deciduous and evergreen species (objective ii), we performed an ANOVA followed by post hoc Duncan test. In order to satisfy the ANOVA test assumptions, data were log or ln transformed when necessary. When transformation was not enough to satisfy the assumptions, a non-parametric test (Kolmogorov-Smirnoff) was carried out. All the statistical analyses were performed using STATISTICA version 7.0 (Statsoft Inc., Tulsa, OK, USA).

Results

Influence of green leaf traits on litter quality

Green leaf traits had a variable influence on senesced leaves depending on specific traits and on functional groups. Structural traits of green leaves have in general a strong influence on structural traits of senesced leaves (R^2 values from 0.6 to 0.96; Fig. 1). Also, deciduous and evergreens responded similarly and did not differ in the slope. The slope of the SMA regressions was not different from 1 in most cases (Fig. 1). Only the relationship of LMA in green and senesced leaves have a slope significantly lower than 1 (slope 0.93). A slope equal to 1 indicates an isometric variation of senesced leaf traits with the green leaf trait changes (Fig. 1). The only difference between functional groups was found in toughness, in which evergreens had a higher elevation shift compared to the deciduous (Fig. 1). This means that, given a certain value of toughness in green leaves. This means that, given a certain value of toughness in green leaves, evergreens had a higher toughness value in senesced leaves than

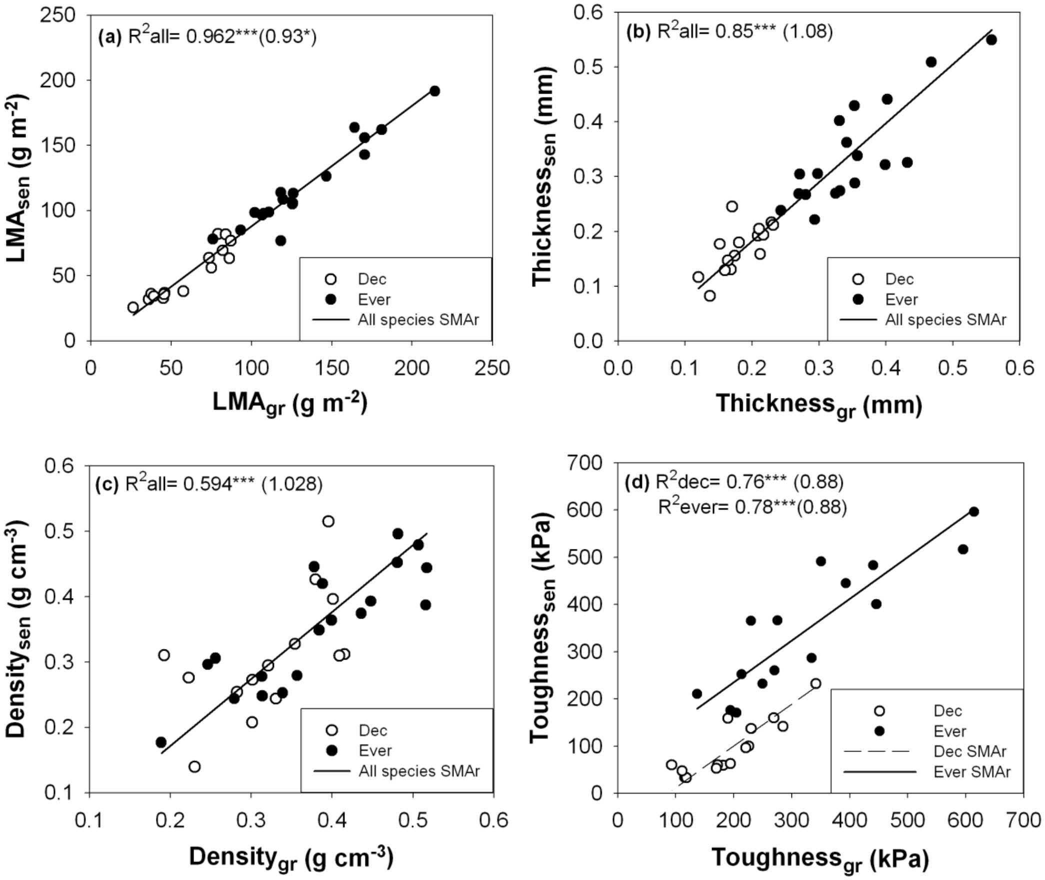

Figure 1. Relationships between traits in green (gr) and senesced (sen) leaves for **a)** Leaf mass per area (LMA); **b)** Thickness; **c)** Density and **d)** Thoughness. The significant SMA regressions are shown with the R^2 and the significance for all, deciduous and evergreen species. The slope of the regression is shown in brackets; asterisks denote a slope significantly different of 1. $P < 0.001$, ***; $P < 0.05$, *.

deciduous.

For C concentration, which is much related to structural traits, there was a strong relationship between green and senesced leaves ($R^2 = 0.78$) and deciduous and evergreens did not differ in the slope (Fig. 2). However, for nutrients (N, P and K) the relations of green and senesced leaves varied depending on the functional group. For example, in deciduous species there was a strong relationship between nutrient concentration (N, P and K) in green and senesced leaves (R^2 from 0.53 to 0.62; Fig.2). In most cases (N and P), the slopes were not different from 1, except for K, where the slope was higher than 1 (slope 1.87), indicating a higher K concentration in senesced leaves with the increasing K concentration in green leaves. However, for evergreens the nutrient concentration in senesced leaves was not closely related to the nutrient concentration in green leaves (Fig. 2). For N concentration, there was a significant relationship of green and senesced leaves, but the slope (0.48) was significantly lower than 1, indicating a lower N concentration in senesced leaves with the increasing N concentration in green leaves. For P and K concentration, there were not significant relationships between senesced and green leaves.

The chlorophyll content per leaf thickness of green and senesced leaves was not significantly related

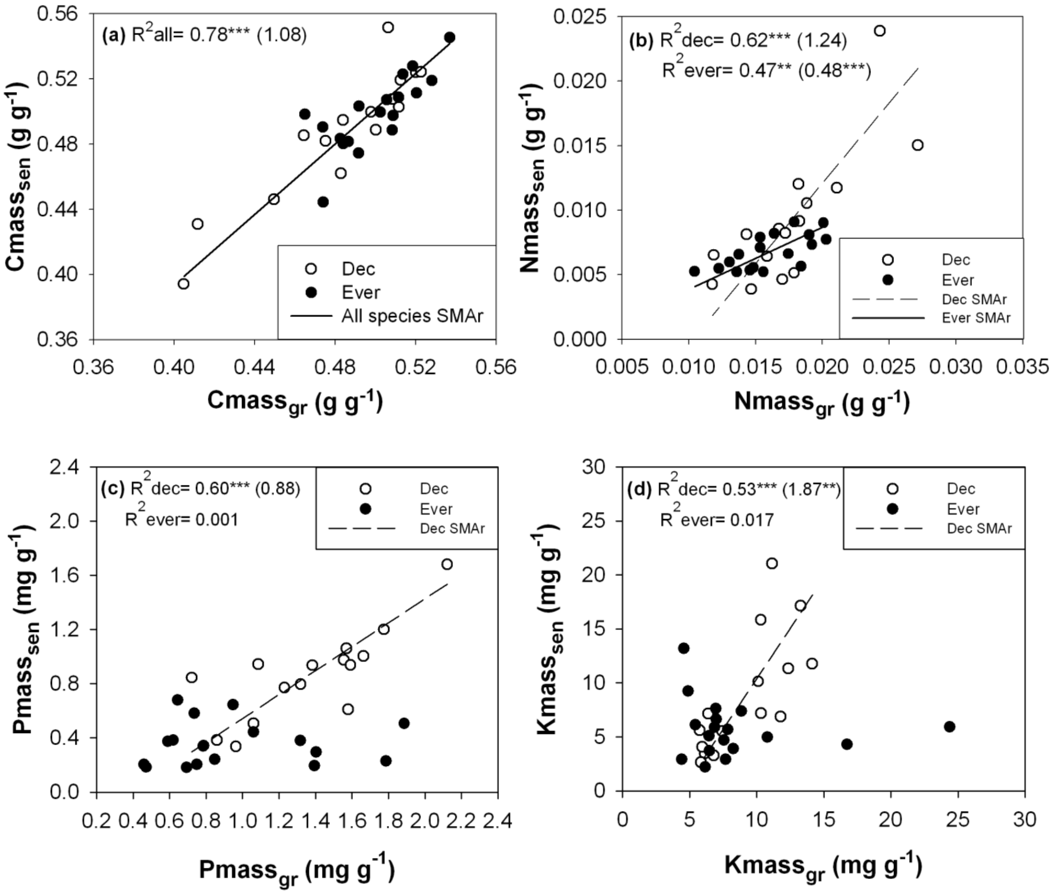

Figure 2. Relationships between traits in green (gr) and senesced (sen) leaves for **a)** C concentration; **b)** Nitrogen concentration; **c)** Phosphorus concentration and **d)** Potasium concentration. The significant SMA regressions are shown with the R^2 and the significance for all, deciduous and evergreen species. The slope of the regression is shown in brackets; asterisks denote a slope significantly different of 1. P < 0.001, ***; P < 0.01, **; P < 0.05, *.

each other (data not shown), since, as expected, the chlorophyll content in senesced leaves was much lower than in green leaves, and most species showed similar values in senesced leaves (except *Alnus glutinosa*, which showed a high chlorophyll content in senesced leaves).

Nutrient resorption efficiency, nutrient proficiency and its relation with leaf traits

The resorption efficiency for all species in the study was lower for C (12.9 ± 9.1 %) and K (23.5 ± 51.6 %) than P (54.3 ± 23 %) and N (59.9 ± 13.2 %; Fig. 3a). Carbon and nutrient resorption efficiencies were equivalent for deciduous and evergreens, except for P, which was higher for evergreens (61.2 ± 22.8 % versus 45.9 ± 20.3 %; Fig. 3a).

Considering all the species, there was not any leaf trait in green leaves (structural or chemical) that can be used as a predictor of carbon and nutrient resorption efficiency (Table S1, Supplementary Information). Only, P resorption efficiency was positively associated to the green-leaves P concentration within evergreens (Fig. 4).

In relation to nutrient proficiency (i.e., the nutrient concentration in senesced leaves), there was not any significant difference between evergreens and deciduous for C, N, P and K concentration (Fig. 3b).

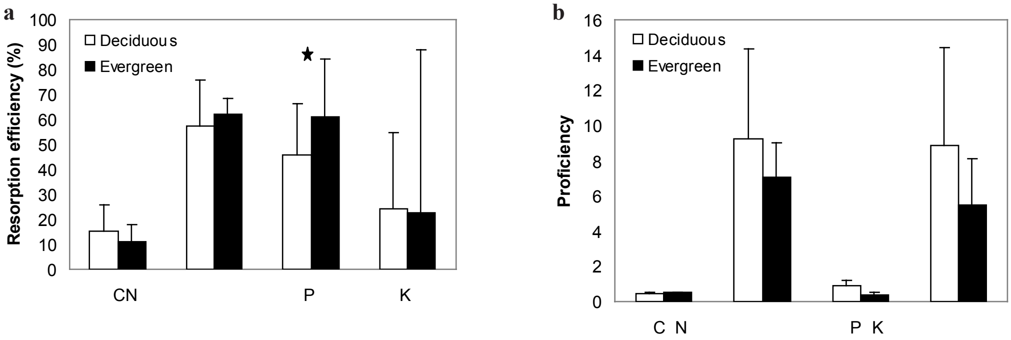

Figure 3. a) Resorption efficiency for C, N, P and K in deciduous and evergreen species. The asterisk denotes a significant difference ($P < 0.05$); **b)** Proficiency (the residual nutrient concentration in senesced leaves) for C, N, P and K in deciduous and evergreen species. Note that the units for C is g g^{-1} and for N, P and K is mg g^{-1}.

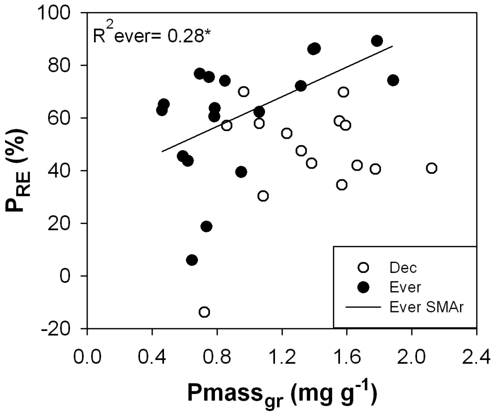

Figure 4. Relationships between phosphorus resorption efficiency (P_{RE}) and phosphorus concentration in green leaves (Pmassgr). The significant regression is shown with the R^2 and the significance level. $P < 0.05$, *.

Discussion

Leaf traits conferring functionality to green leaves (as resistance to herbivory-measured here as leaf toughness-, water retention capacity –as leaf dry matter content- or leaf mass per area, for example) had a strong influence on senesced leaves traits. This indicates that in general, the structural green leaf traits have a key role on litter quality. However, for nutrients (N, P and K) the relationships between green and senesced leaves were dependent on functional groups. Although, for deciduous species there was a strong relationship between nutrients in green and senesced leaves, this was not true for evergreens. Nutrient resorption efficiency was not related to any of the green leaf traits. Plant functional groups, only differed in P resorption efficiency, which was significantly higher for evergreens. We discuss these results along the following sections.

Influence of green leaves traits on litter quality

In our data set, species appeared distributed along a leaf trait spectrum (data not shown), from species with a high nutrient concentration and lower LMA on one extreme (acquisitive strategy), and species with high LMA, low nutrient concentration, high carbon concentration, LDMC, density, thickness and toughness on the other extreme (conservative strategy), as it has been largely described by many authors (Wright and Westoby 2003; Wright et al. 2004; Aponte et al. 2013; Lopez-Iglesias et al. 2014). These two strategies are also related to the ability to grow fast (acquisitive species) and also to the leaf life-span (species with a high nutrient concentration, and the ability to grow fast, also have a short leaf life span; Reich, Walters and Ellsworth 1997).

Looking at senesced leaves (once that the resorption process had taken place), species in our study tended to remain in the same position of the traits continuum previously described by green leaves traits, but only for structural traits. Moreover, the slope in the SMA regressions was, in general, not different from 1, indicating an isometric relationship. Structural traits (toughness, C concentration) have shown to have a strong influence on leaf decomposition (Gallardo and Merino 1993).

However, for nutrients (N, P and K) the relations of green and senesced leaves varied depending on the functional group. For example, in deciduous species there was a strong relationship between nutrient concentration (N, P and K) in green and senesced leaves. However, for evergreens the nutrient concentration in senesced leaves was not strongly related to the nutrient concentration in green leaves. This contrast with the results of other studies, where the nutrient concentration in senesced leaves was positively correlated to that of green leaves (Kobe, Lepzcyk and Iyer 2005; Aponte et al. 2013). This was also found in intra-specific studies (Staaf 1982; Birk and Vitousek 1986; Killingbeck and Costigan 1988).

Evergreens have been found to have lower concentrations in both senesced and green leaves (Kobe, Lepczyk and Iyer 2005), but in our case, this was only true for the P concentration in leaves of evergreen.

A consistency among species in the functional relationship between nutrient concentrations in senesced and green leaves could reflect general biochemical and/or biophysical constraints. For example, a positive linear relationship between nutrient concentrations in senesced and green leaves could arise if nutrient allocation to various biochemical pools (e.g., enzymes, structural proteins) is constant with increased green-leaf nutrient concentration and if pools differ in solubility and/or phloem mobility. In our study, this seemed to be the case for the deciduous, but not for evergreens. This contrasts with the results of Chapin and Kedrowski (1983), who found that evergreens and deciduous species showed similar proportions of N fractions.

Nutrient resorption efficiency and nutrient profficiency

As we mentioned in the introduction section, the values of nutrient resorption efficiency are found to be variable, depending, among other factors, on the proxy used in calculations. It is widely accepted that changes in leaf mass per area (LMA) are possible during senescence as a result of resorption of soluble carbon compounds. Therefore, it is necessary to correct for these changes using a correction factor (van Heerwaarden et al. 2003; Vergutz et al. 2012).

Our estimates of nutrient resorption are in the line of the previous studies. Aerts and Chapin (2000) found that about <5 to 80% of leaf N, and 0–95% of leaf P, may be resorbed, with average values of 52 and 43%, respectively (Chapin and Kedrowski 1983). Also, the meta-analysis from Vergutz et al. (2012) showed resorption percentages as followings: C_{RE} 20-24%; N_{RE} del 41 al 49%; P_{RE} 55-59%, and K_{RE} 41-60%, similar to those found in our study.

Nutrient resorption minimises nutrient losses and therefore high resorption efficiency would be expected from species exhibiting a conservative use of resources. However, we did not find a clear difference in resorption (C, N and K) between deciduous and evergreen species. Only, P resorption efficiency was higher for evergreens. Aponte et al. (2013) did not find any difference in nutrient resorption (N and P) between two species of Quercus (a deciduous and an evergreen). Aerts (1996) observed lower N resorption efficiency in evergreens compared to deciduous (47 vs. 54%, respectively), but not with respect to P (51 versus 50%, respectively). He concluded that the lower nutrient concentration in evergreen leaf-fall contributed more to nutrient conservation than did nutrient resorption.

Kobe, Lepczyk and Iyer (2005) found that N and P resorption efficiency decreased, respectively, with increased N and P green-leaf status. P resorption efficiency and average P concentration in green leaves were inversely related (Pugnaire and Chapin 1993). However, we found the opposite relationship for evergreens, a higher P resorption efficiency with higher P concentration in green leaves.

Kobe, Lepczyk and Iyer (2005) suggest that lower nutrient concentrations in senesced leaves of evergreens may largely reflect lower green-leaf nutrient concentrations resulting from low environmental availability of nutrients, rather than necessarily demonstrating a greater ability by evergreens to draw down leaf nutrient levels prior to abscission as an adaptation to low fertility (cf. Killingbeck 1996; Wright and Westoby 2003). Our results suggest that foliar nutrient concentrations vary continuously and overlap substantially between deciduous and evergreen species (Kobe, Lepczyk and Iyer, 2005).

However, nutrient resorption efficiency did not showed any general pattern in relation to leaf traits. Across variation in nutrient status, consistent allocation to various biochemical pools (e.g., enzymes, structural proteins) of differing solubility and potential for being exported could lead to constant resorption efficiency (Chapin and Kedrowski 1983).

A slope higher than 1 in the relationship between senesced- and green-leaf nutrient concentrations represents decreasing resorption efficiency with increasing green-leaf nutrient concentration, which might be explained by the possibility that, under higher fertility, nutrients may be relatively less expensive to acquire from soil than breaking down foliar compounds, and loading, transporting, and storing breakdown products. In our study, this is only found in K concentration for deciduous species, indicating a lower K resorption efficiency for deciduous with the increasing K green concentration. The opposite result arise for evergreens, which showed slopes lower than 1, indicating a higher nutrient resorption efficiency with the increasing nutrient concentration in green leaves.

Killingbeck (1996) found that evergreens have lower litter P concentrations than deciduous species. He emphasized resorption proficiency (the concentration of a nutrient in senesced leaves) over efficiency because proficiency is not subjected to temporal variation in nutrient concentration in green leaves and timing of sampling. Killingbeck (1996) suggests that selection has minimized nutrient losses through long leaf lifespan and low concentrations of nutrients in senesced leaves, rather than maximizing resorption efficiency. Greater leaf longevity increases nutrient residence time and thus nutrient use efficiency (Berendse and Aerts 1987; Escudero et al. 1992). However, we did not find any difference in nutrient concentration in senesced leaves between deciduous and evergreens.

In summary, we found that the structural green leaf traits have a key role on litter quality and its role is similar for deciduous and evergreens. However, for nutrients (N, P and K) the relationships between green and senesced leaves were dependent of functional groups. For deciduous species there was a strong relationship of nutrients between green and senesced leaves, whereas for evergreens there was not a strong relationship. Functional groups only differed in phosphorus resorption efficiency, which was significantly higher for evergreens. In general, nutrient resorption efficiency was not related to any of the green leaf traits.

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Supporting Information

Table S1. Pearson correlations between nutrient resorption efficiency and chemical composition, structural and physiological traits for all the species (all), deciduous (d) or evergreens (e). Bold font and brown colour indicates a significant correlation ($P < 0.05$), italic bold font and yellow colour indicates a nearly significant correlation ($0.1 > P > 0.05$).

			N_{RE}	C_{RE}	P_{RE}	K_{RE}
Chemical composition traits	**LCC**	**all**	0.20	-0.15	-0.05	-0.05
		d	0.31	0.02	-0.33	0.04
		e	**-0.50**	-0.36	0.02	-0.12
	LNC	**all**	-0.26	0.26	0.06	-0.15
		d	-0.39	0.26	0.02	-0.15
		e	0.32	0.14	0.33	-0.20
	LPC	**all**	-0.17	***0.31***	0.18	0.15
		d	-0.26	0.38	0.21	0.00
		e	0.20	0.10	**0.53**	0.22
	LKC	**all**	-0.06	0.28	0.14	0.29
		d	-0.44	0.06	-0.13	-0.43
		e	**0.50**	***0.47***	0.33	***0.46***
Structural traits	**LMA**	**all**	0.25	-0.15	0.27	0.16
		d	**0.67**	0.03	-0.04	0.38
		e	***-0.42***	0.07	0.04	0.24
	Thck	**all**	0.24	-0.27	0.11	0.03
		d	**0.52**	-0.12	0.07	0.44
		e	-0.06	-0.20	***-0.44***	0.00
	Dens	**all**	***0.33***	0.11	***0.33***	0.20
		d	**0.74**	0.21	-0.09	0.27
		e	-0.34	0.24	***0.42***	0.21
	Tough	**all**	0.24	-0.16	0.08	0.01
		d	***0.47***	-0.19	***-0.45***	0.30
		e	-0.20	0.00	0.06	-0.07
	LDMC	**all**	***0.32***	0.13	***0.31***	0.23
		d	***0.49***	-0.15	-0.30	0.26
		e	0.13	**0.63**	***0.45***	0.26
	LDMCpet	**all**	0.19	-0.24	0.16	0.01
		d	0.28	-0.27	***-0.53***	0.16
		e	**-0.73**	0.23	0.45	0.14
	lmb/pet	**all**	-0.05	-0.06	0.10	0.09
		d	0.02	0.14	-0.31	0.35
		e	-0.50	-0.22	0.27	0.06
Physiological traits	**Chlo/Thck**	**all**	0.01	0.15	***0.35***	-0.12
		d	0.05	0.35	-0.14	-0.09
		e	-0.20	0.29	0.40	-0.14
	Chlo all		0.10	-0.02	**0.39**	-0.12
		d	0.26	0.20	-0.10	0.16
		e	-0.30	0.23	0.35	-0.19

[Fotografía: Castañar de Valdejetas (Córdoba)

Bárbara López]

Capítulo 6. Short-term effects of litter from 21 woody species on plant growth and root development

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REGULAR ARTICLE

Short-term effects of litter from 21 woody species on plant growth and root development

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Abstract

Background and aims Plant litter has an important role in terrestrial ecosystems (Lambers et al. 2008). Our aim was to assess the short-term effect of litter from 21 woody species (deciduous and evergreens) on plant growth and root development.

Methods We conducted a short-term experiment (10 weeks) under controlled conditions adding litter from 21 woody species to pots with *Dactylis glomerata* (target species). We determined plant biomass and root development and related these variables to decomposition rate and litter quality.

Results Litter from two species enhanced plant growth whereas litter of five species inhibited it. Considering all species in the data set, plant growth was associated to litter with high decomposition rate and high litter quality: high Ca and N concentration and low polyphenols concentration. However, excluding from the analyses the two species that increased growth, litter inhibition effect on plant growth was related to the litter-polyphenols concentration. Plants growing with nutrient-richer litter had a lower proportion of fine roots which could be related to a litter mediated increase in soil nutrient.

Conclusions Enhanced plant growth or, on the contrary, plant growth inhibition could be the result of a positive or, in turn, negative balance between nutrient and polyphenols concentration in litter.

Keywords Decomposition rate · Litter calcium · Litter chemical composition · Growth inhibition · Specific root length

Introduction

Plant litter has an important role in terrestrial ecosystems (Lambers et al. 2008). It modifies soil conditions, such as temperature (Heady 1956; Watt 1974), or water content (Zaady et al. 1996; Violle et al. 2006), through litter physical properties. It can also have several effects derived from its chemical composition, such as modifying soil pH (Reich et al. 2005; Koorem et al. 2011), through releasing nutrients (Facelli and Pickett 1991); or nutrient immobilization, by releasing allelopathic compounds (Foster and Gross 1998; Bonanomi et al. 2006; Samedani et al. 2013).

In most cases, effects of litter on plants are assessed looking at total, above-ground biomass or total productivity (Xiong and Nilsson 1999; Boeken and Orenstein 2001; Dorrepaal et al. 2007; Fisher et al. 2013). A few

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studies on litter effects on root growth have been published. Most of these studies showed that litter addition inhibited root growth (Bonanomi et al. 2011; Bughio et al. 2013; Pérez-Corona et al. 2013). In all of them, root growth was measured as root length, root elongation rate or root biomass. However, looking at other root traits as for example root diameter can be also very interesting, given that roots have different functions according to their morphology and size (Eissenstat et al. 2000). Specifically, fine roots (root diameter < 0.5 mm) are mainly involved in soil exploration for water and nutrient absorption, by means of their high surface/volume ratio (Eissenstat 1992; Ostonen et al. 2007).

In addition to root diameter, one of the most frequently measured morphological traits of fine roots is the specific root length (SRL, m g^{-1}). SRL characterize the economic aspects of the root system and it has a fast response to soil conditions. The SRL of fine roots has been shown to increase in response to nutrient limitation (Ryser 2006). Furthermore, as SRL integrates the root length and biomass, it may summarize better the effects of soil conditions on roots.

Xiong and Nilsson (1999), in their metanalysis found that the effects of litter on plant growth were mostly determined by the duration of the study, followed by the ecosystem type and finally to the litter type (evergreen, deciduous trees, grass or forbs). However, most of the variation in plant growth was not explained by these factors, and must be related to others. For instance, litter quality and decomposition rate have been found to be related to litter effects on plant growth. Dorrepaal et al. (2007) found their target plants to have reduced biomass compared to the control when growing with litter with a low initial lignin/P ratio. Moreover, in the second year of study, they found positive effects on plant growth related to litter N concentration and negative ones related to C/N, polyphenol/N and polyphenol/P ratios. Therefore, the short-term effects of litter addition may be different from long-term effects and this seems to be mediated by chemical composition. In this sense, Aponte et al. (2012) revealed that variables controlling litter decomposition and the amount of nutrients released from litter change during decomposition process. In this way, they found litter N and Ca to exert counteractive effects in early versus late decay stages. On the other hand, Bonanomi et al. (2011) found that it is possible to predict litter inhibitory effects across a range of litter types on the basis of their chemical composition, as they found that inhibitory effects of litter on plant growth and root proliferation were most due to the release of allelopathic compounds (i.e. phenolics) than to nutrient inmobilization.

Litter quality and decomposition rate are highly interrelated. Some chemical traits have been described as good indicators of decomposition rate, as the ratio between lignin and carbon and other nutrients (as N or P) (Swift et al. 1979). A high lignin concentration means a high carbon proportion, low palatability for phytophagous and detritivores and low decomposition rate and nutrient release (Melillo et al. 1982; Taylor et al. 1989; Gallardo and Merino 1993; Berg 2000). Nitrogen-rich litter usually decomposes faster than nitrogen-poor litter (Tanner 1981; Köchy and Wilson 1997). On the other hand, the presence of substances as phenolics and tannins or other defensive compounds can slow down the decomposition process (Grime et al. 1996). This is due to the fact that nitrogen forms organic complexes with tannins, instead of being as ammonium, nitrate or amino acids thus decreasing the nitrogen availability for decomposers (Gallardo and Merino 1992; Terradas 2001).

The chemical composition of leaves (Villar et al. 2006) and therefore of leaf litter (Cornelissen 1996; Aerts 1997) is related to leaf habit. Evergreen species, with thick, long-lived and nitrogen-poor leaves (Wright et al. 2004), decompose slower than litter coming from leaves with the opposite traits (Cornelissen 1996; Terradas 2001). Cornwell et al. (2008) in a metanalysis found that woody deciduous species produced litter that decomposed 60 % faster than woody evergreen species. Xiong and Nilsson (1999), in their metanalysis found that deciduous species inhibited plant growth (as aboveground biomass) stronger than those growing with evergreen. This could be due to the faster release of allelopathic compounds from deciduous litter with a high decomposition rate.

On the other hand, litter addition has been traditionally considered to be beneficial for plant growth. For example, in developing countries (i.g. the Himalayan region), litter is widely used as organic fertilizer in agricultural systems and therefore, litter could also play an important role in sustainable development.

All this together highlights the importance to clear up the effects of litter on plant growth and the underlying mechanisms.

In the present study, we assessed the short-term (10 weeks) effect of litter from a wide range of woody species (a total of 21, including deciduous and evergreens) on plant growth and root development of *Dactylis glomerata* (used as a target species) and its relationship with litter quality and decomposition rate, trying to clarify the underlying processes involved.

The aims of this study were: (i) to assess the effects of litter from 21 woody species on plant growth and root development, (ii) to explore the role of decomposition rate and the initial chemical composition of litter on plant growth, (iii) to assess the existence of general patterns of litter effects depending on leaf habit of the species, and (iv) to analyse the root response to increasing nutrient availability in the soil.

Material and methods

Litter and plants preparation

We collected freshly senesced leaves from 21 woody plant species (Table 1), including different leaf habits (evergreen and deciduous) and growth forms (shrubs and trees), in order to cover a wide range of local species. Senescent leaves were collected at Sierra Morena mountains (Córdoba, Spain), covering an area of 8 kms of radius. Deciduous species were collected in the autumn 2011 and evergreen in the spring 2012, as these are the time of litter fall peak for these species. Collection of senescent leaves was done either gently shaking plants or cutting off the leaves that were senescent. Once in the laboratory, litter was air-dried at ambient temperature (about 20 °C) and stored.

To know the effect of litter addition on plant growth, we used *Dactylis glomerata* L. (cocksfoot or orchard grass), which is a perennial grass native from Eurasia. Its distribution is widespread worldwide and is frequent in the Iberian Peninsula. Seeds were obtained from Semillas Cantueso Company (Córdoba, Spain, www.semillascantueso.com). In order to optimize germination, seeds were placed in Petri dishes, between two wet papers, and kept in dark in the fridge (4 °C) for a week. Approximately 208, one-L pots were filled with a nutrient-poor substrate, a mixture of sand and perlite (3:1) (similar to Dorrepaal et al. 2007) and placed in a greenhouse at the University of Córdoba (37° 54′ 48.64″ N, 4° 43′ 17.68″ W) at an altitude of 100 m; (Spain). About 10–15 seeds were placed in each pot. Pots were watered daily during 4 weeks until all plantlets had emerged. Then, four plants per pot were selected for the essay and the rest removed. To assure the same initial conditions before the treatments started, the selected plantlets were cut out to a height of 5 cm.

Growth experiment with litter

About 3 g of fresh, single-species litter were placed in each pot (eight replicates per species) on the surface of the soil. Previously, leaf litters were cut out into 3-cm length pieces to have a better contact with the substrate. Also 16 additional pots with no litter were used as a control. A total of 208 pots (including pots of the fertiliser experiment described below) were placed in the greenhouse distributed in eight trays. These trays were weekly rotated in the glasshouse in order to assure homogeneous conditions for all plants.

All pots were watered by hand to field capacity every other day and a subsample of 50 pots (approximately two pots per treatment) was weighed to know how much water was lost by evapotranspiration and then compensate the water losses by water addition in the same amount. None of the pots lost water due to percolation. The beginning of the experiment was on the 2nd week of May 2012 and it lasted for 10 weeks (end of July 2012). Two measurements of plant height were carried out at 3rd and 7th week to check the effects of litter addition.

Growth experiment with fertiliser

In order to evaluate the response of *D. glomerata* to small increases in the amount of nutrients, a parallel fertiliser experiment was performed. Our objectives were: (i) to test the responsiveness of our target species to small increases in nutrient availability, and (ii) to assess the response of root development to nutrient scarcity.

Nitrogen as NH_4NO_3, and phosphorus as KH_2PO_4 (similar to Dorrepaal et al. 2007) were added, combining three different doses: NP_0, NP_1, and NP_2; which corresponds to different N amount (0, 0.75, 1.5 g, respectively) and P amount (0, 57.7, 115.4 mg, respectively) such that the N: P ratio was around 13 (observed value for Mediterranean species, Gallardo and Merino 1993). We used eight replicates for each dose level. Fertilisers were added to the pots weekly, in small amounts (increasing the percentage of total amount as 5, 10, 15 and 20 % and then decreasing as 20, 15, 10, and 5 %) until they reached the total dose in each treatment.

Table 1 Mean values ± standard error (n for species = 8; n control treatment = 24) for growth variables of *Dactylis glomerata* plants growing with litter of 21 different species and no litter (control treatment)

Litter species	CODE	Family	Leaf habit	Height (cm)	Total root length (m)	Fine root length fraction (%)
Ailanthus altissima Mill.	AA	Simaroubaceae	D	24.85±1.35***	73.38±6.77***	96.16±0.49***
Arbutus unedo L.	AU	Ericaceae	E	10.49±0.91	16.01±2.71	98.21±0.24
Celtis australis L.	CA	Ulmaceae	D	12.68±0.66	20.10±1.89	98.17±0.35
Cistus albidus L.	CAL	Cistaceae	E	11.80±0.62	24.80±2.91	97.66±0.57
Cistus ladanifer L.	CL	Cistaceae	E	9.08±0.79***	8.54±2.31***	99.10±0.12***
Cistus monspeliensis L.	CM	Cistaceae	E	13.79±1.81	16.51±3.48	97.80±0.81
Corylus avellana L.	Cor A	Betulaceae	D	14.34±1.04	15.55±2.46	98.81±0.25
Castanea sativa Mill.	CS	Fagaceae	D	13.58±0.80	22.88±2.78	98.01±0.29
Cistus salviifolius L.	CSA	Cistaceae	E	13.39±0.71	18.97±2.66	98.09±0.29
Ficus carica L.	FC	Moraceae	D	21.47±1.78**	67.49±9.26***	96.86±0.45***
Olea europaea var. sylvestris L.	OE	Oleaceae	E	10.20±0.48	16.08±2.40	98.68±0.25
Phillyrea angustifolia L.	PA	Oleaceae	E	11.21±0.62	15.75±2.26	98.59±0.37
Phlomis purpurea L.	PhP	Lamiaceae	E	14.89±0.81	35.79±2.56	96.78±0.74
Pistacia lentiscus L.	PL	Anacardiaceae	E	14.86±2.49	18.49±1.19	97.73±0.67
Pistacia terebinthus L.	PT	Anacardiaceae	D	14.68±1.04	30.11±4.56	97.53±0.43
Quercus faginea Lam.	QF	Fagaceae	D	13.83±1.54	24.10±2.57	98.28±0.34
Quercus ilex subsp. ballota Samp.	QI	Fagaceae	E	12.35±0.63	21.52±2.55	98.01±0.53
Quercus suber L.	QS	Fagaceae	E	14.89±2.41	27.34±4.52	97.59±0.71
Rhamnus alaternus L.	RA	Rhamnaceae	E	14.59±1.10	15.81±2.23	98.36±0.39
Ulmus minor Mill.	UM	Ulmaceae	D	13.34±0.93	26.41±1.41	97.75±0.35
Viburnum tinus L.	VT	Caprifoliaceae	E	10.96±0.93	9.71±1.96***	98.87±0.18***
No litter	C	–	–	13.60±0.72	26.73±2.36	97.54±0.24
ANOVA (R^2)				47.3***	62.2***	61.9***

Asterisks in the right side indicate the significant effects of the addition of litter of different species on each variable compared to control (no litter) in a post-hoc comparison after a one-way ANOVA among the 22 treatments, following FDR-corrected alpha values ($P<0.014$). Line ANOVA (R^2) shows the variance explained of the one-way ANOVA among treatments. The levels of significance (**, $P<0.01$; ***, $P<0.001$) are indicated

Final harvest

After 10 weeks of experiment, all plants were harvested. Previously, the height of the highest leaf per pot was recorded. Plants were removed from the pot, and separated into above and belowground fractions. Roots were carefully washed and placed on a scanner in a transparent plastic tray filled with water. Total root length (RL_t, m), root diameter (RD, mm) and total root volume (RV, cm^3) were determined using WinRHIZO Pro 2004 (Regent Instruments Inc.). Root length was categorized into three diametrical classes: fine roots (root diameter < 0.5 mm), medium roots (0.5 mm < root diameter < 2 mm) and thick roots (root diameter > 2 mm). Finally, both the roots and the aerial biomass were oven-dried at 70 °C during at least 48 h, weighed, and the shoot-to-root ratio was calculated.

Several root traits describing root structure were calculated, such as specific root length (SRL, root length divided by root dry mass; m g^{-1}), tissue mass density (TMD_r, root dry mass divided by root volume; g cm^{-3}), and root fractions (% length) for each diametrical class (fine, medium and thick roots).

Decomposition experiment

In order to determine the leaf litter decomposition rate of the different species, a parallel decomposition experiment was performed in the same greenhouse at the same time as the growth experiment. Following the standardised methodology (Pérez-Harguindeguy et al. 2013), a subsample of the initial litter was weighed and then oven-dry (60 °C, 48 h) and its dry weight determined. Then, an air-dry mass to oven-dry mass ratio was calculated to estimate the initial dry-weight. The air-dried leaves stored in the laboratory were cut out into 3-cm length pieces as in the growth experiment. Two g of fresh litter were placed in 10×12 cm fibreglass mesh bags (1.5 mm of mesh size). A total of 84 litter bags (4 replicates per 21 species) were prepared. The litter bags were placed in 1-L plastic containers (15.5×6.5×13.5 cm) (hereafter microcosms), with a partially opened lid, to allow for gas exchange. These containers were filled with the same nutrient-poor substrate than in the growth experiment (a mixture consisting of washed river sand and perlite in 3:1 ratio). Each litter bag was buried in substrate (1–2 cm depth), and placed in the greenhouse randomly. In contrast to the growth experiment, this time litter was buried instead of just laid on the substrate surface, in order to keep the litter permanently wet to obtain the maximum decomposition rate. They were all watered up to field capacity at the start of the experiment. Thereafter, microcosms were weighed to monitor water lost by evaporation and compensate those losses by replenishing water in the same amount.

Two bags per species were retrieved from their microcosm after 5 and 10 weeks of experiment. Then, litter was carefully brushed off to remove soil particles, oven-dried at 60 °C for 48 h, and weighed. The initial and the 5 and 10 weeks litter dry weight were used to calculate the percentage of mass loss and the decomposition constant k (see below). The decomposition constant k (Olson 1963) for the exponential relationship was calculated using the equation:

$$\mathrm{Ln}\,(x_1/x_0) = k \times t$$

where x_0 is the original amount of litter, x_1 is the amount of litter remaining after time t, and t is the time (in days). To calculate k, linear regressions of Ln (x_1/x_0) vs. time were performed. The k values represent a 2.5-months period (May 2012 to mid-July 2012, 70 days). Hereafter, we express the decomposition rate in absolute terms for sake of clarity.

Leaf litter analyses

Fresh litter samples were thoroughly mixed before the experiments settings. Then, a sample (one replicate per species) was homogenised using an analytical grinder (Water Cooled Analytical Grinder 2-Blade Cutter, IKA®, Germany). Finally, the litter powder was used to analyse chemical composition. Samples were analyzed for C, N, P, K, Na, Ca, Mg and total polyphenols. C and N concentration were analysed using an elemental analyser (Eurovector EA 3000; EuroVector SpA, Milan, Italy). About 0.25 g of litter powder was digested in nitric/perchloric acid and the resulting solution analyzed for Ca and Mg by atomic absorption spectrophotometry using an atomic absorption spectrometer (Aanalyst 200; Perkin Elmer Inc., USA). K and Na in the solution was analysed by flame emission with a flame photometer (Allen et al. 1976) (Jenway, Bibby Scientific Limited, Staffordshire, UK); and P with the molybdenum blue colour method of Murphy and Riley (1962) and measured using an UV/VIS spectrometer (Lambda 35, Perkin Elmer Inc., USA). Polyphenols were extracted using aqueous acetone (70 %) and determined using the Folin-Ciocalteau method with tannic acid (from Merck®) as standard (Makkar and Becker 1993). Total polyphenols were expressed as tannic acid equivalent (TA). The amount of available *Phillyrea angustifolia* fresh litter, was not enough to perform the digestions, therefore its nutrient concentration (P, K, Mg, Ca, Na and polyphenols) were not determined.

The nutrient and polyphenols concentrations were expressed on an oven-dry weight basis, using the air-dry mass to oven-dry mass ratio determined in a subsample of the initial litter. This proxy was used to preserve the fresh chemical composition of litter samples.

Data analyses

One-way ANOVA analyses were performed to test for differences in growth variables (we used one ANOVA test per each variable) among treatments [21 species of litter + control (no litter) = 22 treatments]. Then, a multiple comparison of means test (*post hoc* Unequal *N* Tukey's Honestly Significant Difference test) was carried out, looking at differences of species of litter to the control. In order to control the inflation of type I error derived from repeated testing, the false discovery rate (FDR, the expected proportion of tests erroneously declared as significant) criterion was applied to the ANOVA tests. The FDR was controlled at the 5 % level using a standard step-up procedure (see García 2004). Data were logtransformed when necessary to fulfil the ANOVA assumptions. In addition, to test for the effects of the different leaf habit of the litter species (deciduous vs. evergreen) on plant variables, one-way ANOVA analyses were also performed per each variable, followed by a multiple comparison of means test (*post hoc* Unequal *N* Tukey's Honestly Significant Difference test).

The same approach was used to test for the effect of fertiliser addition (NP_0=control, NP_1, NP_2) on growth variables, and to compare the litter chemical composition among leaf habit (deciduous vs evergreen). For these tests the FDR criterion was not applied to the ANOVA tests, given the low number of tests.

Pearson correlations were used to evaluate the relationships among variables (decomposition rate and fresh litter initial chemical concentrations to plant growth and root development variables). A principal component analysis (PCA) was carried out to summarize the chemical composition variables in one (called Factor 1 PCA).

All analyses (except ANOVA) were performed twice: 1) considering all species of litter, and 2) without considering litter from *Ailanthus* and *Ficus* (those that showed a positive effect on plant growth). Litter from these two species performed quite different from the rest so that we explored general patterns by excluding them from the analyses. All the statistical analyses were performed using STATISTICA version 7.1 (Statsoft Inc., Tulsa, OK, USA).

Results

Litter addition has a different effect depending on species

Whole plant level

There were differences in plant height among treatments (Table 1). Plants growing with *Ailanthus* and *Ficus* litter developed higher plants compared to the control (no litter). However, *C.ladanifer* litter had a negative effect on plant height, resulting in shorter plants compared to the control.

Total, aboveground and belowground biomass were also different among plants growing with litter of different species (Fig. 1; Table S1, Supplementary Material). As with plant height, plants with litter of *Ailanthus* and *Ficus* yielded a higher biomass (above, belowground and total) than the control treatment. On the other hand, plants with litter from *Arbutus*, *C. ladanifer*, *Phillyrea*, *Rhamnus* and *Viburnum* had a lower biomass (either total aboveground and/or belowground) when comparing to the control plants (Fig. 1; Table S1, Supplementary Material). No differences in the shoot: root ratio were found among litter species or comparing to the control (shoot:root average of 1.52) (Table S1, Supplementary Material).

Root variables

Plants growing with *Ailanthus* and *Ficus* litter showed a higher total root length and a lower proportion of fine roots than the control plants (Table 1). However, plants growing with *C. ladanifer*, and *Viburnum* litter showed a lower total root length and a higher proportion of fine roots than the control plants.

In general, root traits did not differ among plants growing with different litter and control plants. For the specific root length (SRL), we found differences among plants growing with different litter species, but not compared to the control (Table S1, Supplementary Material). For the tissue mass density (TMDr), none of the differences were statistically significant (Table S1, Supplementary Material).

Remaining biomass, decomposition rate and initial element concentration

Litter from different species differed strongly in their percentage of remaining biomass after 10 weeks of decomposition. Species as *Ailanthus*, *Rhamnus*, *Phlomis* and *Ficus* reached approximately 40–50 % of remaining biomass in 10 weeks, while species as *Q. faginea*, *Q. ilex*, *Q. suber*, *Castanea* and *P. terebinthus* kept about 80 % of the initial biomass in the same period (data not shown). Thus, the species with highest decomposition rates were *Ailanthus*, *Rhamnus*, *Phlomis* and *Ficus* ($[k]\approx 0.010\ day^{-1}$), whereas *Q. faginea*, *Q. ilex*, *Q. suber*, *Castanea*, and *P. terebinthus* had the lowest values ($[k]=0.002\ day^{-1}$; data not shown).

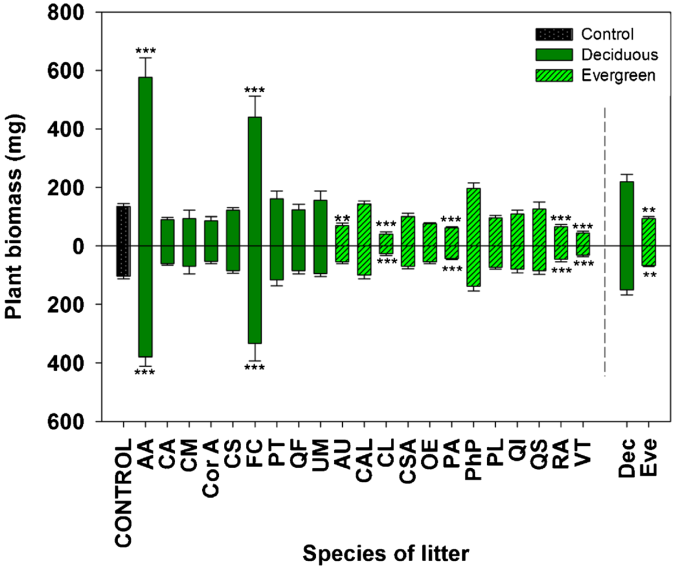

Fig. 1 Aboveground and belowground biomass of *Dactylis glomerata* cultivated with litter of 21 different species and control (no litter). For species codes see Table 1. Bars on the left of the vertical line represent the mean value and standard error of each species of litter, while the mean and stardard error of all deciduous and all evergreen species are shown after the vertical line. Black bars represent the control treatment, while smooth-dark green bars are the deciduous species, and shaded-bright green bars correspond to evergreen. Asterisks indicate significant differences to the control in the post-hoc comparison in a one-way ANOVA among treatments [21 species of litter + control (no litter) = 22 treatments], following FDR-corrected alpha values ($P<0.014$). Also the results of the post-hoc comparison of the ANOVA test among leaf habit (Dec + Ever + Control = 3 treatments) are shown. The levels of significance (**, $P<0.01$; ***, $P<0.001$) are indicated

Different species differed in their initial litter chemical composition (data not shown). Some N poor species were *C. ladanifer* or *Arbutus* (about 6.5–8.5 mg g^{-1}) whereas N rich species were *Ailanthus* and *Phillyrea* (about 16–20 mg g^{-1}). Phosphorus concentration ranged from 0.2 mg g^{-1} (*P. terebinthus*) to 1.9 (*P. lentiscus*). Calcium concentration ranged from around 7 to 32 mg g^{-1}, for *C. monspeliensis* and *Ailanthus* respectively. Total polyphenols content ranged from 8 to 122 mg TA eq. g^{-1} (tannic acid equivalent) for *Ficus* and *C. ladanifer*, respectively. The C:N ratio ranged from 25 to 75, being most species around 30–60 (data not shown). The two first axes of the PCA analysis explained ca. 60 % of the variation of the initial chemical composition (Fig. 2). The first axis, which explained the 39 % of the total variation, was positively related to total polyphenols, and negatively to Ca concentration. The second axis was positively related to Mg and negatively related to P concentration. When litter from *Ailanthus* and *Ficus* were not considered in the PCA, the two first axes of the PCA explained a 51 % of the variation of the initial chemical composition (data not shown), and the first axis, which explained a 30 % of the total variation, was positively related to total polyphenols and negatively to sodium concentration (data not shown).

Relationships among decomposition rate, litter quality and plant response

The decomposition rate was related to the concentration of some nutrients. For example, K and Ca initial litter concentrations were positively correlated to the decomposition rate (Table 2). In the case of Ca, the correlation disappeared when litter from *Ailanthus* and *Ficus* was not considered in the analysis.

The litter decomposition rate was positively related to biomass production (Fig. 3a) (either above and belowground or total biomass), plant height and total root length, but all these positive relationships disappeared when *Ailanthus* and *Ficus* were excluded from the data set (Table 2). Litter decomposition rate showed a negative trend ($P<0.10$) with the proportion of fine roots only when considering all species (Table 2).

Total biomass was positively related to initial litter concentrations of Ca (Fig. 3b) and N, and showed a

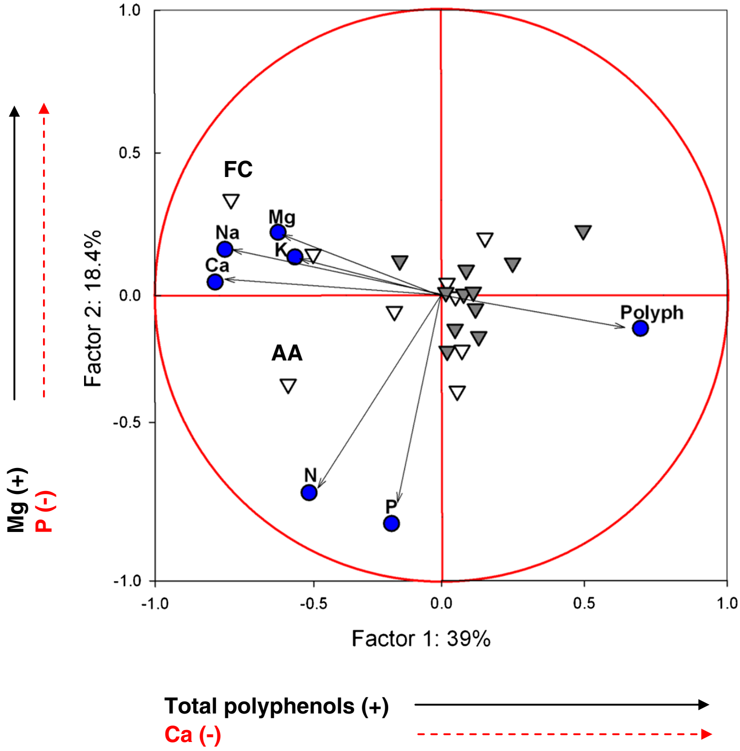

Fig. 2 Principal component analysis (PCA) for initial chemical composition of litter from 21 woody species. *Blue circles* represent the loadings for 7 traits on the first two PCA axes, while *inverted triangles* indicate the scores of the species in the factor plane. *Filled triangles* are for evergreen species and empty for deciduous. The *arrows* indicate the four variables most related to the two main factors and the direction of the relationship. The position of *Ailanthus* (AA) and *Ficus* (FC) is shown

Table 2 Pearson correlation coefficient for the decomposition rate (k; day^{-1}, in absolute values), growth and root development variables with the initial element concentration and total polyphenols (tannic acid equivalents) of litter from 21 different species. (°0.05<P<0.1; * P<0.05; ** P<0.01;*** P<0.001). Blue values show the coefficient when litter from *Ailanthus* and *Ficus* were not considered

		Initial element concentration (mg g^{-1}d.w.)							
	k (day^{-1})	N	P	K	Ca	Mg	Na	Total polyphenols	C: N
k (day^{-1}) (without AA, FC)		0.28 -0.38	0.07 0.03	0.73 *** 0.67 **	0.50 * 0.06	0.40 ° 0.26	0.13 -0.03	-0.06 0.22	-0.11 0.12
Height (cm) (without AA, FC)	0.54 * 0.02	0.59 ** 0.20	0.24 0.40 °	0.37 0.04	0.79 *** 0.38	0.49 * 0.15	0.40 ° 0.38	-0.49 * -0.40	-0.43 ° -0.38
Total biomass (g) (without AA, FC)	0.54 * -0.17	0.50 * 0.04	0.05 0.01	0.43 ° 0.16	0.80 *** 0.32	0.43 ° -0.40	0.40 ° 0.50 *	-0.49 * -0.48 *	-0.31 -0.11
Total root lenght (m) (without AA, FC)	0.49 * -0.19	0.44 ° 0.06	0.01 -0.06	0.40 ° 0.11	0.81 *** 0.40 °	0.42 ° -0.40 °	0.43 ° 0.42 °	-0.54 * -0.54 **	-0.31 -0.16
SRL (m g^{-1}) (without AA, FC)	-0.19 0.34	-0.34 0.14	-0.28 -0.35	-0.32 -0.05	-0.52 * 0.09	-0.26 0.23	-0.33 -0.23	0.15 -0.15	0.11 -0.13
Fine root lenght fraction (%) (without AA, FC)	-0.40 ° 0.05	-0.42 ° -0.04	-0.16 -0.16	-0.40 ° -0.19	-0.67 ** -0.32	-0.19 0.29	-0.40 ° -0.35	0.49 * 0.36	0.30 0.13

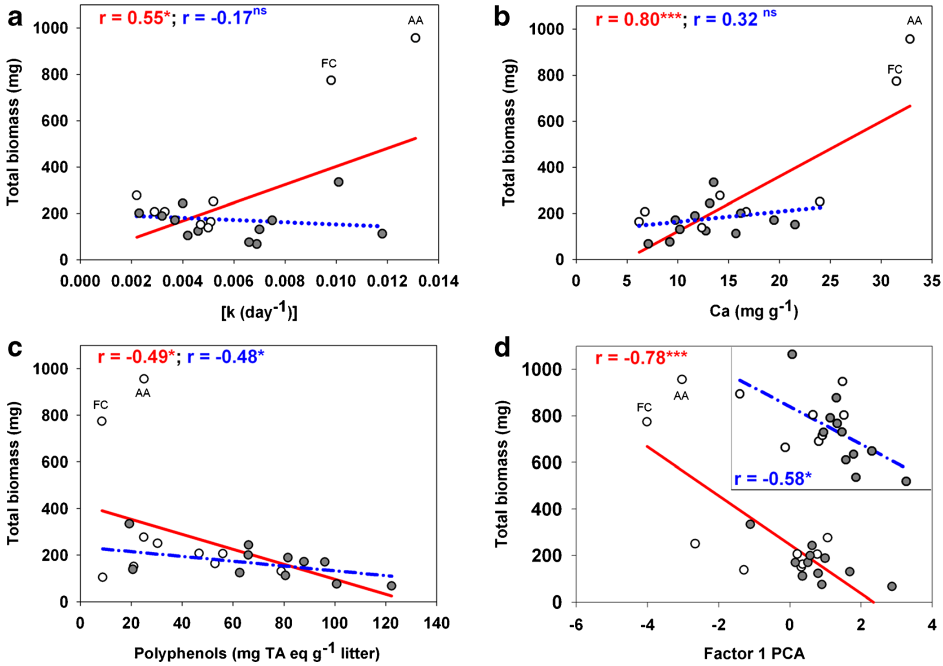

Fig. 3 Relationships between total plant biomass and **a** decomposition rate, **b** initial litter calcium concentration, **c** polyphenols concentration and **d** the factor 1 of the PCA (which summarizes all chemical composition variables in one, see Material and Methods). Regression lines, Pearson correlation coefficient (r), and significance levels are shown (* $P<0.05$; *** $P<0.001$). *Blue-dotted lines* show the relation when litter from *Ailanthus* and *Ficus* are not considered. *Dash-dotted lines* indicate a significant relation, whereas *dotted lines* indicate not significant relation. The first r-value (in red) is the coefficient considering all species whereas the second r-value (in blue) was calculated without considering litter from *Ailanthus* or *Ficus*. In Fig. 4d, the up-right box show the relation when the PCA was performed without considering litter from *Ailanthus* or *Ficus*. Deciduous: empty symbols, evergreens: filled symbols

positive trend ($P<0.10$) with K, Mg and Na (Table 2). Plant height and total root length showed the same correlations with nutrients as well (Table 2). However, all these correlations disappeared or were weakened when *Ailanthus* and *Ficus* were omitted in the analysis.

On the other hand, total biomass, plant height and total root length were negatively related to total polyphenols concentration (Table 2, Fig. 3c), being this relationship consistent after removing *Ailanthus* and *Ficus* data from the analysis (Fig. 3c). The factor 1 of the PCA was negatively correlated to total biomass (Fig. 3d) for both analyses (with and without *Ailanthus* and *Ficus* data).

Fine root length fraction and SRL were negatively related to litter Ca only when considering all species (Table 2). Fine root length fraction showed a negative trend ($P<0.1$, Table 2) with N, K and Na concentrations. The factor 1 of the PCA was positively related to SRL (Fig. 4), but this relationship disappeared when *Ailanthus* and *Ficus* data were omitted.

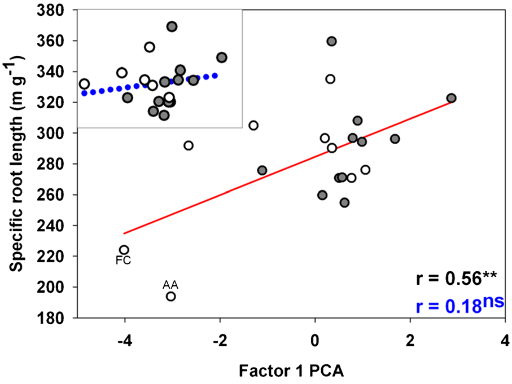

Fig. 4 Relationship between specific root length (SRL) and the factor 1 of the PCA. The up-left box show the relation when the PCA was performed without litter from *Ailanthus* or *Ficus*. The *blue-dotted line* indicates not significant relation. Deciduous: empty symbols, evergreens: filled symbols

Effect of leaf habit on plant growth

There were differences between plants growing with litter from species with distinct leaf habits. Plants growing with litter of evergreen species had a lower biomass (aboveground, belowground and total biomass) than those growing with litter of deciduous species or control plants (Table 3; Fig. 1). Also, plants growing with evergreen litter were shorter and had a lower total root length than plants growing with deciduous litter, but they were not different from the control. No differences were found for SRL and any other root traits (Table 3). In addition, looking at litter chemical composition, we found that evergreen species had a lower Ca and Na concentration, and also a higher total polyphenols concentration (Table 3).

Differences among treatments (control, deciduous and evergreen) remained the same when excluding data of *Ailanthus* and *Ficus* from the analyses excepting plant height (which was the same for the three groups), and total root length (plants growing with evergreen litter were different from deciduous litter and control treatments). When data of *Ailanthus* and *Ficus* were removed from the analyses, the only difference in litter chemical composition was the highest total polyphenols concentration in litter from evergreen species (data not shown).

Fertiliser experiment

Plants growing with fertiliser showed a 15-fold increase in total biomass and were 3 times taller when comparing to the control treatment (NP_0, no fertiliser) (Table S2, Supplementary Material). Plants treated with fertiliser also showed a higher shoot/root fraction (Fig. 5a), a lower specific root length (Fig. 5b), a higher proportion of medium-size roots (Fig. 5c), and lower proportion of fine roots than control plants (Table S2, Supplementary Material).

Discussion

Litter addition showed different effects depending of the different species of litter used, from null to negative or

Table 3 Effect of litter type (deciduous, evergreen litter and control) on plant growth, root development variables and litter chemical composition (element concentrations in mg g^{-1} of dry weight)

		Control (no litter)	Deciduous litter	Evergreen litter
Growth	Height (cm)	13.6±0.72 ab	16.01±0.66 a	12.5±0.39 b
	Aboveground biomass (mg)	134.68±9.85 a	219.21±25.31 a	94.38±5.37 b
	Belowground biomass (mg)	104.04±8.52 a	150.93±17.48 a	66.67±4.21 b
	Total biomass (mg)	238.72±17.47 a	370.15±42.21 a	160.41±9.4 b
Root development	Total root length (m)	26.73±2.36 ab	35.00±3.07 a	18.87±0.98 b
	Avg. Root Diam. (cm)	0.19±0.00	0.19±0.00	0.18±0.00
	SRL (m g^{-1})	271.32±11.24	274.07±9.21	297.31±7.53
	TMDr (g cm^{-3})	0.14±0.01	0.14±0.00	0.15±0.00
	Fine root length fraction (%)	97.54±0.24	97.69±0.16	98.11±0.14
	Medium root length fraction (%)	2.45±0.24	2.30±0.16	1.88±0.14
	Thick root length fraction (%)	0.006±0.002	0.004±0.001	0.004±0.001
Litter chemical composition	Carbon	–	485.11±19.04	506.41±5.95
	Nitrogen	–	12.82±1.16	11.37±0.84
	Phosphorus	–	0.79±0.14	0.92±0.12
	Potasium	–	7.43±2.08	8.86±1.3
	Calcium	–	19.99±3.26 a	12.08±1.12 b
	Magnessium	–	2.34±0.57	1.8±0.25
	Sodium	–	0.21±0.05 a	0.11±0.02 b
	Polyphenols	–	29.15±5.4 a	71.06±8.65 b

Mean values and standard error (N=8 for deciduous; 13 for evergreen) are shown. Different letters indicate significant differences ($P<0.05$) in a post-hoc comparison after a one-way ANOVA among leaf habit

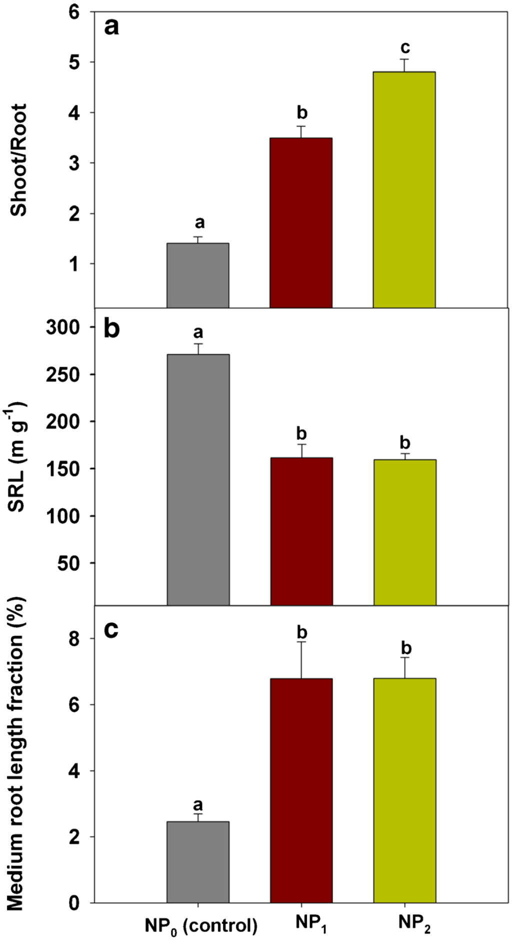

Fig. 5 Effect of fertiliser addition on **a** shoot/root ratio, **b** specific root length (SRL) and **c** medium root fraction (in length). Different letters above columns indicate significant differences between treatments ($P<0.05$). To check for the different doses of fertiliser (NP$_1$ and NP$_2$), see Material and Methods

positive effects. Among species that affected plant growth, only 2 species (*Ailanthus* and *Ficus*) promoted it whereas many more (5 species) inhibited it. The two litter species that enhanced plant growth (both deciduous) were characterised by a high decomposition rate and a high litter quality (i.e. high Ca and N concentration and low total polyphenols concentration). On the other hand, the five evergreen species that inhibited plant growth had high total polyphenols concentration. In addition, our results showed that increases in nutrient availability affected root traits in our target species, which also responded to polyphenols concentration producing shorter roots. Moreover, roots growing under fertilisation conditions were thicker (higher diameter, lower SRL, lower proportion of fine roots) and longer than those growing in poor-nutrient conditions (control plants). In the following sections, we discuss these results in more detail.

Effects of litter addition on plant growth

Despite the short-term effects of litter on plants have been described as negative in a meta-analysis using data from 35 independently published studies world-wide (Xiong and Nilsson 1999), we have found litter coming from our species to exert both, positive and negative effects on plant growth.

Litter enhancing plant growth (*Ailanthus* and *Ficus*) showed a high decomposition rate, high Ca and N concentration (in the case of *Ailanthus*), and low total polyphenols concentration (especially *Ficus*). Although it would be expected that nutrient-rich litter with high decomposition rates, could enhance nutrient availability (Demey et al. 2013), and therefore, plant growth, we did not find a consistent relationship across all species between decomposition rate or litter nutrient concentration and total plant biomass. Only the litter Ca concentration showed a strong positive relationship with plant biomass that disappeared when data from *Ailanthus* and *Ficus* were removed from the data set. Thus, we hypothesize that in the case of litter coming from these two species, the positive effect on plant biomass could be in part explained by the release of Ca. Note that the Ca concentration was much higher in *Ailanthus* and *Ficus* than in any other species (the mean value for these two species was 31 mg g^{-1}, whereas the mean value for the rest was 13 mg g^{-1}). Although this can not be postulated as an overall pattern across species in our study, litter Ca has been encountered to modify some soil properties leading to improved plant productivity. For instance, four-fold variation in leaf-litter Ca concentration among 14 tree species was linked to variation in soil pH, and exchangeable Ca (Dauer et al. 2007). Moreover, our target species has shown an enhanced plant growth, as a response to Ca addition in a sandy substrate (Crossley and Bradshaw 1968). In the mentioned essay, aimed to compare the growth response of several populations of *Dactylis* to increasing levels of Ca, all populations responded with an increase in plant biomass. Thus, a 27-fold increase in the calcium added to sand (from 2 to

54 ppm) led to a 50 % rise on plant biomass (Crossley and Bradshaw 1968).

Litter N concentration was also related to plant biomass but it was neither consistent when data of *Ailanthus* and *Ficus* were removed from the analyses. Based on the results of our fertiliser experiment (N and P addition to the soil increased plant growth in relation to the control treatment), one might expect that a high N and P concentration in litter would have the same enhancing growth effect (Foster and Gross 1998). However, we did not find such effect, which can be due to the low rate of N addition coming from the litter. If we compared the highest amount of N potentially released by litter with the minimum dose used in the lowest fertiliser treatment (NP_1; 750 mg of N), we still find differences of one order of magnitude: litter from *Ailanthus*, the nitrogen-richest species of litter, could potentially release a maximum of 60 mg of N (20 mg g^{-1} × 3 g of litter added). The low amount of N coming from litter decomposition, together with the presence of growth inhibiting compounds (polyphenols) could explain why we did not find a strong relationship between N concentration in litter and plant growth. A similar reasoning can be applied to P concentration.

In addition, plant biomass and total root length were negatively correlated to the litter total polyphenols concentration. This correlation was the only consistent relationship that remained significant when data of *Ailanthus* and *Ficus* were removed from the data set. Thus, we believe that the total polyphenols concentration in litter was a very important variable affecting plant growth in our study.

Polyphenols have been recognized as regulators of soil processes, inhibiting nitrification, as well as decomposition rates (Verhoeven and Toth 1995) and nutrient recycling (Hättenschwiler and Vitousek 2000; Joanisse et al. 2007). They are also known to affect litter quality, through physico–chemical effects on the pools and forms of nutrients (Hättenschwiler and Vitousek 2000). Even more, it has been described that some species are capable of degrading fertile soils through production of polyphenols-rich litter, sequestering soil nutrients into unavailable form and creating unfavourable conditions for seed germination, root growth, and nutrient uptake (Northup et al. 1998).

Therefore, our results suggested that enhanced plant growth or, on the contrary, plant growth inhibition could be the result of a positive or, in turn, negative balance between nutrient and total polyphenols concentration in litter. Accordingly, growth inhibition could be associated to the release of allelopathic compounds (Bonanomi et al. 2006; Dorrepaal et al. 2007), and the potential underlying mechanisms for enhanced plant growth could consist in a small polyphenols disruption and a litter mediated increase in soil nutrient availability (Facelli and Pickett 1991).

Regarding all those species that showed no effects on plant growth, we hypothesize that it could be associated to several factors, such as a low decomposition rate, a high nutrient immobilization by microorganisms, or to a neutral balance between negative (high total polyphenols concentration) and positive effects (litter mediated nutrient increase in the soil). Nutrient immobilization by saprophytic microbes may outcompete plants for this limiting resource, resulting in a low availability for plants of nutrients derived from leaf litter decomposition. This process is likely to occur in decaying plant tissues with a high C/N ratio (Hodge et al. 2000). Initial tannin content also has been found as a significant predictor of the maximum N immobilization (Gallardo and Merino 1992).

Effects of litter on root development

Several effects of litter addition to root development have already been described. For instance, it has been found that when plants are grown in poor-nutrient environments, the assignment to the belowground fraction increases, this leads to a decrease of shoot/root ratio and also roots tend to increase their uptake capacity (Terradas 2001; Bonanomi et al. 2011). Thanks to the fertiliser experiment, we could confirm that our target species responded to increases in nutrient availability and that plants responded to nutrient scarcity producing thinner roots (higher SRL; higher proportion of fine roots) and a lower shoot-to-root ratio (Fig. 5a and b; Table S2 Supplementary Material). It has been found that enhanced nutrient availability under fertilization reduces the need for the investment in explorative fine roots and thus account for a decrease in SRL (Ostonen et al. 2007). Contrary to expectations, we did not find any differences of shoot:root ratio among different species of litter in our growth experiment, but, in turn, we observed a higher proportion of fine roots in those plants growing with high polyphenols concentration litter (*C. ladanifer* or *V. tinus*). This suggest, not a higher allocation to roots as a response to low nutrient concentration (as shoot:root ratio did not show any differences among different litter species), but a greater production or investment in fine roots in the presence of growth-inhibiting compounds as polyphenols. We also found a significant negative correlation between SRL and total biomass, ($r=-0.78$; $P<0.0001$), that remained the same when data of *Ailanthus* and *Ficus* were removed from the data set. This relation indicate that smaller plants, growing with litter with high total polyphenols concentration had also less developed roots, related to their lower total plant size. These results could be interpreted as a plastic change of roots in response to a litter-mediated inhibitory effect on roots development and therefore on whole plant growth. Thus, plants were smaller in size and under-developed in their ontogeny stage when comparing to the control. However, it should be noted that our results were based in a short-term study, and the observed effects may change over time (Xiong and Nilsson 1999). A shift in the effect of litter on plant growth was described by Bonanomi et al. (2011) where the undecomposed litter inhibited *Lepidium sativum* root growth, but this inhibitory effect disappeared 30 days later.

In our study, those plants growing with Ca-poor litter also had higher values of SRL (Table 2). Calcium in soil is known to have an important role, given that it corrects soil acidity, and raises the pH values, because it is a non-hydrolysing (i.e. "base") cation that competes with H^+ and Al^{3+} cations for exchange sites on soil particle surfaces (Duchaufour 1984; Reich et al. 2005). Litter and soil Ca concentration and pH can also potentially influence C and N cycling because higher pH is associated with greater microbial biomass and higher rates of litter decomposition, soil respiration and net N mineralization (Reich et al. 2005). Furthermore, Ostonen et al. (2007), in a review about SRL data for different European tree species found that the SRL of *Picea abies* resulted negatively correlated with soil pH. In an essay with two *Quercus* species, to evaluate how host trees modify abiotic conditions through their leaf fall quality, (Aponte et al. 2010) found some very high correlations ($r>0.9$) between litter Ca, soil pH and soil Ca concentration. Furthermore, in a study with litter from 14 tree species, differences in litter Ca resulted in profound changes in soil acidity and fertility (Reich et al. 2005). We suggest that the greater Ca release from calcium-rich litter may modify substrate pH, raising it. This could explain that plants growing with Ca-poor litter produced a higher proportion of fine roots, and, furthermore, roots with a higher SRL.

Effects of leaf habit

Regarding our searching for general patterns of effects depending on leaf habit of the species of litter, we found that litter of evergreen species inhibited plant growth. Similar results were found by Koorem et al. (2011) who found higher biomass of their target species (herbaceous plants) below a deciduous shrub (*Corylus avellana L.*) compared to the evergreen species (*Picea abies L.*), which had poor litter quality (low nutrient concentrations and high levels of secondary chemicals).

In our experiment, we found that litter from evergreen had lower Ca concentration and higher total polyphenols concentration than deciduous species. In general, Mediterranean woody evergreens make a great investment in the biosynthesis of carbon-based-secondary compounds rather than investing in nitrogen-based compound biosynthesis (Villar et al. 2006; Di Ferdinando et al. 2013). Furthermore, some polyphenols compounds are considered involved in secondary cell wall thickening (Di Ferdinando et al. 2013). Consequently, the litter from evergreen species is described as thick and nitrogen-poor litter, and decompose slower than those with opposite traits, retaining N in the organic matter for longer periods and therefore limiting primary production in ecosystems with dominance of these species (Terradas 2001). In addition, Aponte et al. (2010) found that litter and topsoil sampled under the canopy of a winter deciduous species (*Q. canariensis*) were significantly richer in Ca, and the soils were less acidic than those sampled under the evergreen oaks. Moreover, they also found that initial Ca in litter was 1.5-fold higher in the deciduous oak than in the evergreen (*Q. suber*). Also, litter-Ca in *Q. canariensis* was released faster than in *Q. suber* (Aponte et al. 2012).

Our results indicate that inhibition of plant growth observed with litter coming from evergreen litter is more related to its higher total polyphenols concentration than to its nutrient status, although, the negative balance between calcium concentration and polyphenols should not be discarded.

In a global change scenario, if species shifts induced by climate change favored evergreen species or species with high polyphenol concentration, then a primary productivity decrease could be expected, based on the results of this and other experiments (Dorrepaal et al. 2007).

Conclusion

The effect of litter on plant growth differed among species. The main factor related to litter effects on plant growth seemed to be the litter polyphenols concentration, as a high polyphenols concentration coincided with plant growth inhibition whereas species with enhanced plant growth had a low polyphenols concentration. In addition, we found that the two species that enhanced plant growth also had a high litter Ca concentration. Thus, we hypothesize that a positive balance between Ca concentration and polyphenols could be the underlying mechanism explaining the positive effect of these two species on plant growth in our experiment. The no effect on plant growth observed in most species could be also a consequence of the balance between polyphenols and Ca concentrations. Plants growing with litter from *Ailanthus* or *Ficus* had a lower proportion of fine roots, which could be related to a litter mediated increase in soil nutrient. It is possible that plant litter had an inhibitory effect on root development and thus on whole plant growth.

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Electronic Supplementary Material.

Short-term effects of litter from 21 woody species on plant growth and root development. *Plant and Soil.*

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Table S1. Mean values ± standard error (n for species = 8; n control treatment=24) for growth and root variables of *Dactylis glomerata* plants growing with litter of 21 different species and no litter (control treatment): total biomass, shoot/root, average root diameter, specific root length (SRL), root tissue mass density (TMDr) and medium root length fraction Marks in the left column indicate the significant effects of the addition of litter of different species on each variable compared to control (no litter) in a post-hoc comparison after a one-way ANOVA among the 22 treatments, following FDR-corrected alpha values ($P < 0.014$). Line ANOVA (R^2) shows the variance explained of the one-way ANOVA among treatments.The levels of significance (**, $P < 0.01$; ***, $P < 0.001$) are indicated.

Litter species	Code	Family	Leaf habit	Total biomass (mg)	Shoot/Root	Avg. Root Diameter (cm)	SRL (m g^{-1})	TMDr (g cm^{-3})	Medium root length fraction (%)
Ailanthus altissima Mill.	AA	Simaroubaceae	D	*** 955.96 ± 91.53	1.53 ± 0.15	0.21 ± 0.61	193.71 ± 9.89	152.80 ± 6.90	*** 3.83 ± 0.49
Arbutus unedo L.	AU	Ericaceae	E	** 123.93 ± 15.24	1.32 ± 0.21	0.18 ± 0.36	296.63 ± 30.77	145.54 ± 14.60	1.79 ± 0.24
Celtis australis L.	CA	Ulmaceae	D	150.95 ± 12.38	1.53 ± 0.09	0.18 ± 0.48	335.02 ± 23.14	126.48 ± 9.60	1.83 ± 0.35
Cistus albidus L.	CAL	Cistaceae	E	243.04 ± 22.34	1.68 ± 0.22	0.18 ± 0.67	254.79 ± 15.08	152.78 ± 11.16	2.34 ± 0.57
Cistus ladanifer L.	CL	Cistaceae	E	*** 67.44 ± 12.59	1.67 ± 0.08	0.16 ± 0.27	322.58 ± 22.03	154.30 ± 12.37	*** 0.90 ± 0.12
Cistus monspeliensis L.	CM	Cistaceae	E	163.50 ± 53.70	1.66 ± 0.14	0.18 ± 1.12	290.15 ± 39.15	152.83 ± 23.06	2.19 ± 0.80
Corylus avellana L.	Cor A	Betulaceae	D	138.43 ± 20.56	1.72 ± 0.10	0.17 ± 0.46	304.84 ± 25.76	153.79 ± 11.17	1.19 ± 0.25
Castanea sativa Mill.	CS	Fagaceae	D	206.49 ± 17.44	1.44 ± 0.37	0.18 ± 0.49	270.69 ± 22.13	150.59 ± 10.27	1.98 ± 0.29
Cistus salviifolius L.	CSA	Cistaceae	E	170.98 ± 18.67	1.47 ± 0.52	0.18 ± 0.42	270.69 ± 21.25	149.29 ± 10.27	1.91 ± 0.29
Ficus carica L.	FC	Moraceae	D	*** 774.24 ± 130.35	1.36 ± 0.13	0.20 ± 0.54	224.03 ± 20.03	152.57 ± 12.15	3.14 ± 0.45
Olea europaea var.sylvestris L.	OE	Oleaceae	E	130.71 ± 8.42	1.82 ± 0.09	0.17 ± 0.40	296.00 ± 26.37	160.44 ± 13.80	1.31 ± 0.25
Phillyrea angustifolia L.	PA	Oleaceae	E	*** 104.23 ± 7.54	1.53 ± 0.34	0.17 ± 0.49	366.37 ± 22.96	128.93 ± 11.71	1.41 ± 0.37
Phlomis purpurea L.	PhP	Lamiaceae	E	334.35 ± 35.13	1.47 ± 0.06	0.20 ± 0.84	275.58 ± 25.01	127.75 ± 7.89	3.21 ± 0.73
Pistacia lentiscus L.	PL	Anacardiaceae	E	170.20 ± 12.16	1.35 ± 0.06	0.19 ± 0.89	259.64 ± 21.33	153.26 ± 15.02	2.26 ± 0.66
Pistacia therebintus L.	PT	Anacardiaceae	D	277.15 ± 45.41	1.46 ± 0.12	0.19 ± 0.64	275.97 ± 25.64	139.99 ± 12.68	2.47 ± 0.43
Quercus faginea Lam.	QF	Fagaceae	D	206.96 ± 30.54	1.45 ± 0.12	0.18 ± 0.47	296.56 ± 25.19	142.10 ± 9.87	1.72 ± 0.34
Quercus ilex subsp. ballota Samp.	QI	Fagaceae	E	188.89 ± 25.37	1.50 ± 0.07	0.18 ± 0.74	294.23 ± 25.99	141.96 ± 13.72	1.99 ± 0.53
Quercus suber L.	QS	Fagaceae	E	200.35 ± 35.43	1.50 ± 0.13	0.19 ± 1.02	271.05 ± 38.21	147.49 ± 12.10	2.41 ± 0.70
Rhamnus alaternus L.	RA	Rhamnaceae	E	*** 112.09 ± 14.61	1.54 ± 0.12	0.17 ± 0.56	359.41 ± 22.42	122.20 ± 8.83	1.64 ± 0.38
Ulmus minor Mill.	UM	Ulmaceae	D	250.99 ± 35.28	1.70 ± 0.16	0.18 ± 0.46	291.73 ± 23.74	132.82 ± 6.63	2.25 ± 0.35
Viburnum tinus L.	VT	Caprifoliaceae	E	*** 75.69 ± 11.44	1.54 ± 0.10	0.17 ± 0.34	307.87 ± 13.56	150.98 ± 8.16	*** 1.13 ± 0.18
No litter	**C**	-	-	238.72 ± 17.47	1.41 ± 0.13	0.19 ± 0.33	271.32 ± 11.24	140.45 ± 8.31	2.45 ± 0.24
ANOVA (R^2)				74.4***	5.28 ns	30.8***	25.3***	9.1 ns	54.7***

Table S2. Effect of two fertiliser doses on plant growth, biomass allocation and root development. Letters on right indicate significant differences between treatments ($P < 0.05$).

		NP_0 (control)	NP_1	NP_2
Growth	**Height (cm)**	13.6 ± 0.72 a	40.41 ± 2.22 b	39.79 ± 2.38 b
	Aboveground biomass (mg)	134.68 ± 9.85 a	2526.06 ± 87.12 b	2905.94 ± 212.48 b
	Belowground biomass (mg)	104.04 ± 8.52 a	752.75 ± 67.57 b	612.01 ± 52.91 b
	Total biomass (mg)	238.72 ± 17.47 a	3278.81 ± 136.55 b	3517.95 ± 256.87 b
Biomass allocation	**Shoot/ Root**	1.41 ± 0.13 a	3.49 ± 0.24 b	4.81 ± 0.25 c
Root development	**Total root length (m)**	26.73 ± 2.36 a	124.26 ± 18.14 b	97.55 ± 9.58 b
	Avg. Root diam. (cm)	18.73 ± 0.33 a	25.68 ± 1.55 b	26.68 ± 0.7 b
	SRL ($m\ g^{-1}$)	271.32 ± 11.24 a	161.53 ± 14.26 b	159.29 ± 6.6 b
	TMDr ($g\ cm^{3}$)	0.14 ± 0.01	0.13 ± 0.01	0.11 ± 0.01
	Fine root length fraction (%)	97.54 ± 0.24 a	93.18 ± 1.13 b	93.17 ± 0.64 b
	Medium root length fraction (%)	2.45 ± 0.24 a	6.78 ± 1.11 b	6.79 ± 0.63 b
	Thick root length fraction (%)	0.01 ± 0.00 a	0.04 ± 0.02 b	0.03 ± 0.01 b

Capítulo 7. Discusión general

El objetivo general de esta tesis es conocer si los rasgos funcionales (de hoja, tallo y raíz) de las plantas están asociados a distintos procesos, respuestas y estrategias de las especies en distintas fases de su vida (plántula y adulto). Para ello se han combinado estudios observacionales con experimentos en condiciones controladas (invernadero). Con esto nos proponíamos comprobar si dichos rasgos funcionales pueden usarse como predictores de tales procesos y respuestas, de forma que pudieran proporcionar información de los futuros cambios de las especies en un escenario de cambio global.

En líneas generales, en esta tesis se ha estudiado:

(i) la relación de los rasgos funcionales con el crecimiento en plántulas en invernadero y adultos en campo;

(ii) los rasgos funcionales y la respuesta a la sequía y la distribución de las especies a lo largo de un gradiente de disponibilidad hídrica;

(iii) los cambios que se producen en las raíces muy finas como respuesta a la sequía y si tales cambios determinan una mayor tolerancia a la misma;

(iv) la influencia de los rasgos de la hoja verde sobre la calidad de la hojarasca y la eficiencia en la reabsorción de nutrientes;

(v) el efecto de los rasgos de la hoja y la descomposición de la hojarasca sobre el crecimiento de las plantas.

Un esquema de los principales resultados de esta tesis puede verse en la Fig. 1. A continuación se detalla una discusión general acerca de ellos:

Crecimiento y su relación con los rasgos funcionales en seis especies de Quercus

El crecimiento de las plantas se puede definir como el balance positivo entre la captura y las pérdidas de carbono, nutrientes y agua (Lambers et al. 2008). Las plantas actúan a la vez como fuente y sumidero de carbono (Dixon et al. 1994) y por ello es crucial conocer cómo funcionan las plantas, ya que el CO_2 es un gas con efecto invernadero que está aumentando exponencialmente debido a la actividad humana.

La mayoría de los trabajos en condiciones de campo estiman el crecimiento basado en incrementos en altura y/o en diámetro del tronco, considerándose que son buenas estimas del crecimiento en biomasa.

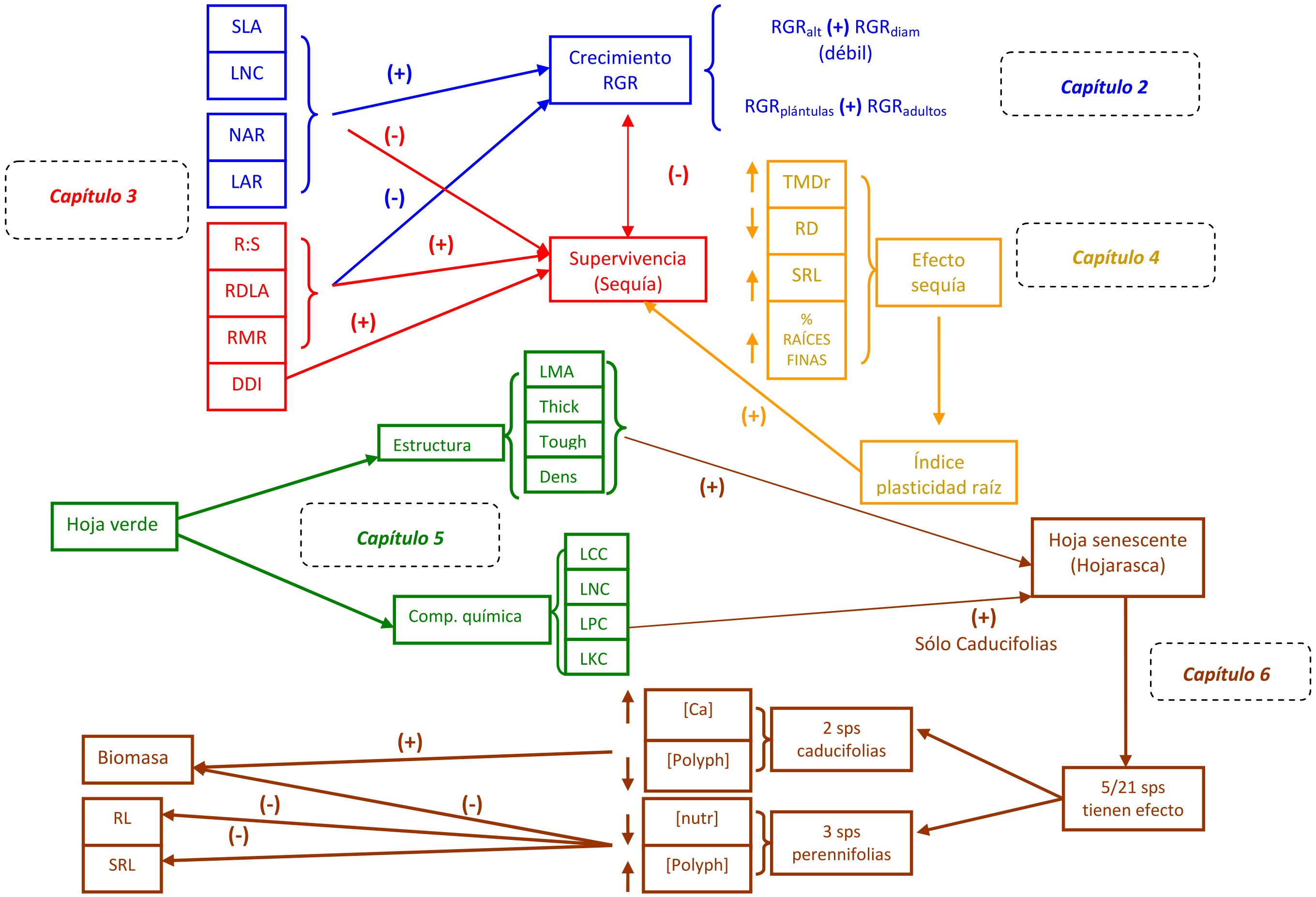

Figura 1. Relación entre los principales rasgos vegetales y los distintos procesos de las plantas estudiados en esta tesis, así como los capítulos en los que se estudia cada uno de ellos. Principales resultados de esta tesis.

Por otro lado, los valores de crecimiento obtenidos en condiciones controladas y en plántulas podrían diferir de aquellos medidos en condiciones de campo y en adultos. No existen trabajos que comparen diferentes tasas de crecimiento de distintas especies de *Quercus* en condiciones controladas y en campo.

Por todo esto, sería muy interesante conocer a qué rasgos funcionales se asocian las diferencias en el crecimiento entre especies y entre plántulas y adultos de una misma especie.

Nuestros resultados (Capítulo 2) indican que la tasa de crecimiento relativo (relative growth rate, RGR) de plántulas de especies del género *Quercus* es más baja en general que la del resto de especies usadas en el estudio. Estas diferencias parecen deberse principalmente al área específica foliar (specific leaf area, SLA), un rasgo funcional muy asociado con la tasa de crecimiento (Fig.1; Cornelissen et al. 1996; Antúnez et al. 2001; Ruiz-Robleto y Villar 2005). Las hojas de *Quercus* tienen un valor bajo de área específica foliar (SLA), que las hace ser especies de tipo "conservativo" (Díaz et al. 2004; Wright et al. 2004).

Por otro lado, las especies de *Quercus* también tienen una baja razón de área foliar (leaf area ratio, LAR) y una baja tasa de asimilación neta (net assimilation rate, NAR). Lo cual puede deberse en parte a que las especies de *Quercus* presentan una gran proporción de biomasa en las raíces (45.0 ± 3.0 %) frente a otras especies leñosas (25.0 ± 1.4 %). Una mayor proporción en raíz puede ser una ventaja para resistir la sequía en climas mediterráneos, al tener acceso a una mayor cantidad de agua en el suelo, resultando en mayores tasas de supervivencia (Lloret et al. 1999). En *Quercus*, la fuerte inversión en raíz hace que la proporción de biomasa dedicada a las hojas sea muy baja en comparación a otras especies.

Se podría esperar que las tasas de crecimiento expresadas en altura y diámetro (variables que se miden de forma sistemática en los inventarios forestales) fuesen similares. Sin embargo, la relación entre el crecimiento relativo en altura y en diámetro fue muy baja (Fig.1). A pesar de que para todas las especies se encontró una relación positiva y significativa, el porcentaje de explicación de una variable sobre otra fue muy bajo (del 1 al 2 % dependiendo de la especie). Esto sugiere que hay grandes diferencias entre el crecimiento en altura y diámetro, y que por tanto las conclusiones basadas en crecimiento en altura pueden diferir de las basadas en diámetro. Por otro lado, el grado de precisión en la estima de la altura en especies con copas irregulares hace del cálculo de la RGR en altura un parámetro con un grado de error superior al de RGR en diámetro. En resumen, un árbol puede crecer presentando un fuerte incremento en altura pero cambiando poco el diámetro (ver Fig. 4, Capítulo 2), lo cual, según los predictores de Montero et al. (2005) indicarían un crecimiento nulo. Por ello, nuestros resultados indican la importancia de estimar la biomasa considerando tanto incrementos de altura como en diámetro, y que además podrían variar de una manera importante dependiendo de las condiciones climáticas y estructurales de la parcela.

La tasa de crecimiento relativo en árboles estuvo muy relacionado con la tasa de crecimiento relativo en plántulas (Fig.1, Discusión general; Fig. 7 A, Capítulo 2), aunque hay que resaltar la diferencia de unidades: en plántulas de 10 a 20 mg g^{-1} $día^{-1}$ y en árboles de 16 a 30 mg g^{-1} $año^{-1}$), debido posiblemente a las limitaciones de recursos y condiciones que se encuentran en condiciones naturales.

Es de destacar también cómo dos rasgos funcionales importantes de la hoja como el SLA y la concentración de N presentaron una relación muy buena (R^2 de 0.69 a 0.85) con el crecimiento de los árboles en condiciones naturales y podrían considerarse como buenos predictores de las tasas de crecimiento (Fig.1; ver e. g. Ruiz-Benito et al. 2014).

Rasgos funcionales asociados a la sequía en el mediterráneo

La disponibilidad de agua es un factor limitante clave para las plantas en muchos ambientes, que afecta a su crecimiento y supervivencia (Ciais et al. 2005; Bréda et al. 2006) y, por tanto, a la distribución de las especies (Engelbrecht et al. 2007; Valladares, 2008). En los ecosistemas mediterráneos, el establecimiento de las plántulas en los primeros estadíos es especialmente crítico, dado que, durante el verano, una sequía prolongada e intensa coincide con el período más cálido del año (Quero et al. 2008; González-Rodríguez et al. 2011). Además, las previsiones para las próximas décadas, indican que la región mediterránea de la Península Ibérica se enfrenta a una disminución del 20% en las precipitaciones y un aumento de 2-3 °C en la temperatura (MARM 2009), lo que limitará aún más la disponibilidad de agua.

Las plantas han desarrollado diversas estrategias para hacer frente a la sequía (Chaves et al. 2002; Valladares 2008). Las razones por las que algunas especies resisten a la sequía mientras que otras sucumben, aún no se conocen completamente (McDowell et al. 2008). Varios rasgos de raíz, tallo y hoja parecen ser responsables de los diferentes niveles de tolerancia al estrés por sequía. Los diferentes rasgos funcionales pueden asociarse, configurando distintas estrategias frente a la sequía, que pueden explicar la distribución de las especies en la naturaleza.

Nuestros resultados (Capítulo 3) indican que las diez especies leñosas mediterráneas difirieron fuertemente en su respuesta a la sequía, mostrando una gran diferencia en su tiempo de supervivencia. Encontramos dos estrategias claramente contrastadas. Por un lado, tenemos aquellas especies que crecen más rápido, y tienen una tasa de asimilación neta elevada. Este primer grupo está formado por especies de hoja caduca que producen hojas baratas de construir (es decir, con un alta área específica foliar -SLA- y con baja concentración de C), y presentan una relación raíz/ parte aérea alta. También tienen raíces profundas, pero un área transpirante muy elevada [y por lo tanto, un cociente profundidad raíz/área foliar (RDLA) bajo; Fig.1. Estas especies mostraron un tiempo de supervivencia muy bajo, y se encuentran distribuidas de forma natural en sitios relativamente húmedos, como indica su bajo índice distribución con respecto a sequía (drought distribution index, DDI; Fig.1). Este primer grupo está formado por especies *adquisitivas* (*sensu* Díaz et al. 2004), cuya estrategia consiste en el aprovechamiento de los recursos disponibles para crecer rápidamente, siempre y cuando las condiciones y recursos sean favorables. Están por tanto limitadas a sitios relativamente ricos en recursos (agua y nutrientes). El segundo grupo está constituido por especies de hoja perenne (*Q. suber*, *Q. ilex* y *Pinus*), que se caracterizan por una tasa de crecimiento relativo lenta, una tasa de asimilación neta baja y hojas caras de construir (esclerófilas y con alta concentración de C). Construyen raíces profundas y tienen un área foliar baja, lo que favorece la exploración del suelo para buscar agua y minimiza las pérdidas de agua por transpiración. Estas especies tienen un tiempo de supervivencia mayor (Fig. 1). Este segundo grupo está formado por especies *conservativas* (*sensu* Díaz et al. 2004).

En general, encontramos un compromiso entre un crecimiento rápido y la supervivencia a la sequía (Fig. 1), que está en línea con el compromiso crecimiento versus supervivencia encontrado para otros recursos limitantes en otros sistemas. Por ejemplo, en un estudio (Poorter y Bongers 2006) con 53 especies de la selva tropical, que difieren en su tolerancia a la sombra, se encontró que la tasa de crecimiento relativo (RGR) estaba negativamente correlacionada con la supervivencia a la sombra. De forma similar, Sánchez-Gómez et al. (2006) encontraron que las especies leñosas mediterráneas con una alta RGR (principalmente *Quercus* caducifolios) mostraron una supervivencia moderada a la sequía.

La distribución de las especies según la disponibilidad de agua (DDI) estuvo estrechamente asociada con la concentración de C de la hoja. Las especies de los sitios más secos (mayor DDI) tenían hojas más esclerófilas y ricas en carbono. Esto podría explicarse por el hecho de que las hojas esclerófilas tienen alto contenido en hemicelulosa y lignina (Robichaux et al. 1986) y una cutícula gruesa y son típicas de especies mediterráneas, que están, en general, adaptadas a las condiciones secas (Villar et al. 2006).

La supervivencia a la sequía mostró una correlación positiva con el índice DDI (Fig. 1), lo que apoya nuestra hipótesis. Sin embargo, el tiempo de supervivencia explicó sólo un 42% de la distribución de las especies en el campo, lo que sugiere que la tolerancia a sequía no es el único factor que configura la distribución de estas especies mediterráneas, aunque tal vez es el más importante. De forma similar, Engelbrecht et al. (2007) encontraron que la sensibilidad a la sequía fue un buen predictor de la distribución de las especies a lo largo de gradientes de disponibilidad de agua locales y regionales. Otros factores que covarían con la disponibilidad de agua (por ejemplo, la disponibilidad de luz y nutrientes, o la presión de herbívoros y patógenos), pueden también afectar a los patrones de distribución de las especies (Engelbrecht et al. 2007). En resumen, nuestros resultados sugieren que las estrategias frente a la sequía pueden explicar la distribución de las especies en ambientes mediterráneos, aunque otros factores (como la temperatura, el uso del suelo, la disponibilidad de nutrientes del suelo y la resistencia al fuego) también pueden ser importantes (Valladares 2008).

Los rasgos funcionales de la raíz cambian con la sequía

La capacidad de las plantas para modificar las características de sus raíces puede ser un mecanismo muy importante para evitar el estrés por sequía. La mayoría de los estudios de sequía se centran en las respuesta de la parte aérea de las plantas y menos en las raíces, debido principalmente a las dificultades que entraña la observación y el estudio de estas últimas (Huang y Gao 2000; Poorter et al. 2012). Las raíces representan del 20 – 40 % de la biomasa total de la planta (Jackson et al. 1996; 1997; Antúnez et al. 2001; Alameda y Villar 2009; Poorter et al. 2012). Por ello, es interesante conocer como responden las raíces finas de distintas especies a un evento severo de sequía.

La sequía provoca que las plantas inviertan más en raíz y menos en tallo (Quero et al. 2006; Poorter et al. 2012), y también afecta a la distribución vertical de las raíces, de manera que éstas puedan acceder a capas más profundas donde la disponibilidad de agua es mayor durante la sequía (Padilla y Pugnaire 2007; Markejstein y Poorter 2009).

Las raíces tienen distintas funciones según su diámetro (Eissenstat et al. 2000), concretamente las raíces muy finas (diámetro <0.5 mm) tienen un papel clave en la exploración del suelo en busca de agua y nutrientes, debido a su alta relación superficie/volumen (Eissenstat 1992; Ostonen et al. 2007). La sequía puede cambiar la estructura y función de las raíces finas, que están descritas por rasgos funcionales clave, como la longitud específica de la raíz (specific root length, SRL, m g^{-1}), que indica cuanta longitud de raíz puede fabricarse por unidad de masa (Kramer y Boyer 1995; Ryser 2006).

La plasticidad de las raíces cuando los recursos son limitantes está considerada un aspecto importante de adaptación de las plantas (Grime 1994; Grime y Mackey 2002). La sensibilidad a la variación en la humedad del suelo y la capacidad para una respuesta plástica puede ser crucial en plantas que viven en hábitats sujetos a estrés hídrico (Bell y Sultan 1999).

En el capítulo 4, observamos que la sequía incrementa la proporción de biomasa destinada a las raíces. Esto ha sido observado en más estudios, con un incremento de moderado (Padilla et al. 2009; Poorter et al. 2012) a fuerte (Poorter et al. 2012), dependiendo de la intensidad de la sequía.

Aunque la mayor parte de la biomasa de raíz se encontró en los 10 primeros cm (debido posiblemente a que la mayoría de los nutrientes se suelen encontrar en los primeros cm de suelo, Ho et al. 2005), la sequía provocó un cambio en la distribución vertical de las raíces hacia capas más profundas, lo que podría favorecer la adquisición de agua en zonas más profundas del suelo (White y Castillo 1989; Ho et al. 2005). Las raíces profundas y la distribución vertical de las raíces son rasgos de adaptación a la sequía (Passioura 1983; Taiz y Zeiger 2006; Padilla y Pugnaire 2007).

Además, la sequía provocó un incremento en la proporción de raíces muy finas (< 0.5 mm) en los horizontes más profundos y esto estuvo relacionado con una mayor supervivencia (Fig. 1). Esto pudo deberse a un cambio en las estructura de las raíces o bien a la proliferación de nuevas raíces más finas como respuesta a la menor disponibilidad de agua en el suelo, tal y como sugieren el incremento en la longitud específica de la raíz (SRL) y la disminución del diámetro de la raíz (RD; Fig. 1). Algunos estudios han encontrado que el SRL es más bajo en plantas sometidas a sequía (Baburai 2006; Ebrahim 2008), aunque no se encontró un efecto claro de la sequía en el SRL en la revisión de Ostonen et al. (2007). En general, tal y como muestra nuestra revisión, el efecto de la sequía sobre el SRL es muy variable. Esto puede deberse a que el SRL depende de la densidad y el diámetro de la raíz (Wright and Westoby 1999; Nicotra et al. 2002; Ostonen et al. 2007), que pueden tener respuestas muy distintas a la sequía (Ostonen et al. 2007).

Una ventaja que obtendría la raíz al disminuir su diámetro como respuesta a la sequía, es que los vasos xilemáticos al ser de menor diámetro (Fitter 1987) podrían ser más resistentes al embolismo que puede provocar la sequía (Alameda y Villar, 2012).

La resistencia a la sequía puede ser mayor mejorando la capacidad de las raíces para extraer agua del suelo (Wright et al. 1994), lo que puede venir determinado por cambios en el SRL y otros rasgos relacionados. En este estudio encontramos cambios en los rasgos de la raíz (SRL, TMDr, diámetro de la raíz) como respuesta a la sequía (Fig. 1), lo que demuestra una respuesta plástica de las raíces (Sultan 1995). Las especies con un mayor índice de plasticidad en los rasgos de la raíz sobrevivieron más tiempo a la sequía (Fig. 1). Este resultado está de acuerdo con Grime, (1994), que concluye que la capacidad para modificar el sistema radicular para mantener las funciones vitales y el crecimiento cuando los recursos son limitados puede ser un aspecto clave de la plasticidad adaptativa de las especies.

La reabsorción de nutrientes y su relación con los rasgos foliares

La reabsorción de los nutrientes procedentes de las hojas senescentes es un mecanismo de conservación de nutrientes muy importante, que permite a las plantas usar los mismos nutrientes repetidas veces (Aerts 1996; Wright y Westoby 2003; Yasumura et al. 2005) y ser así menos dependientes de la disponibilidad de nutrientes del suelo cuando éstos escasean (van Heerwaarden et al. 2003). La reabsorción de nutrientes también es importante porque puede determinar la calidad de la hojarasca y por tanto su tasa de descomposición, lo que afectaría a la liberación de nutrientes al suelo y finalmente a la fertilidad del mismo (Norris et al. 2012; Aponte et al. 2013).

La eficiencia en la reabsorción se ha relacionado con el estatus nutricional de la planta (a mayor concentración de nutrientes en la planta, menor eficiencia, Lajtha 1987). También se ha asociado con la fertilidad del suelo, de manera que se ha considerado que aquellas especies en suelos más pobres tendrán una reabsorción más eficiente (Vitousek 1982; Ralhan y Singh 1987). Sin embargo, también se ha encontrado la relación contraria a ésta (Chapin y Kedrowski 1983; Lajtha 1987; Nambiar y Fife 1987), o que la eficiencia en la reabsorción no cambia con fertilidad del suelo (Millard y Nielsen 1989; Schlesinger et al. 1989; Chapin y Moilanen 1991).

Por otro lado, se han buscado relaciones entre rasgos funcionales de la hoja y la eficiencia de reabsorción. Wright y Westoby (2003) encontraron que las especies con un alto peso específico foliar (leaf mass per area, LMA) también tienen una baja concentración de nutrientes en las hojas verdes y también una baja concentración residual de nutrientes en las hojas senescentes (Killingbeck 1996). Sin embargo, Wright y Westoby (2003) no encontraron ninguna correlación entre la reabsorción de nutrientes propiamente dicha y los rasgos foliares.

Proponemos dos hipótesis mutuamente excluyentes, en relación a la reabsorción de nutrientes y los rasgos funcionales y grupos funcionales. Por una parte, podríamos esperar que las especies caducifolias, que son metabólicamente más activas que las perennifolias, podrían también conseguir una remobilización de nutrientes más eficiente, y por tanto tener una mayor eficiencia en la reabsorción de nutrientes. Por tanto, como las caducifolias tienen un LMA más bajo que perennifolias podríamos esperar una relación negativa entre la eficiencia en la reabsorción y el LMA.

Por otra parte, también podría ocurrir que las perennifolias, que son especies de carácter más conservativo y tienen concentraciones de nutrientes más bajas (Wright y Westoby 2003; Villar et al. 2006) fueran más eficientes reabsorbiendo nutrientes, dado que la reabsorción de nutrientes es un mecanismo de conservación de nutrientes (Aponte et al. 2013).

Los resultados del capítulo 5 indican que los rasgos estructurales de la hoja verde (como LMA, el grosor, la densidad o la resistencia a la rotura) tuvieron una gran influencia sobre los rasgos de las hojas senescentes (Fig. 1). Se ha descrito que rasgos como la concentración de C o la resistencia a la rotura, ambos estructurales, tienen una gran influencia sobre la tasa de descomposición de la hojarasca (Gallardo y Merino 1993).

Sin embargo, para los nutrientes, la relación entre las hojas verdes y senescentes dependió del grupo funcional. Así, para caducifolias, la concentración de nutrientes (N, P y K) en la hoja verde fue determinante para la concentración en las senescentes, mientras que en perennifolias no hubo ninguna relación o fue mucho más débil (Fig. 1). Esto contrasta con los resultados de otros estudios, donde la concentración de nutrientes de la hoja verde sí estaba relacionada con la de la hoja senescente en perennifolias (Aponte et al. 2013; Kobe, Lepzcyk y Iyer 2005). Esto podría deberse al grupo de especies elegido para nuestro estudio, en el que puede no haber ninguna tendencia o patrón.

Los grupos funcionales sólo difirieron en la eficiencia de reabsorción del fósforo, que fue significativamente mayor para perennifolias. En general, en contra de nuestra hipótesis inicial, la eficiencia de reabsorción de nutrientes no estuvo relacionada con ninguno de los rasgos foliares estudiados.

Influencia de la calidad de hojarasca sobre el crecimiento

La hojarasca tiene un papel fundamental en los ecosistemas terrestres (Lambers et al. 2008), y sus efectos sobre las plantas pueden ser muy importantes. La hojarasca modifica las condiciones del suelo, como la temperatura (Heady 1956; Watt 1974), o el contenido de agua (Zaady et al. 1996; Violle et al. 2006). También puede modificar el pH del suelo (Reich et al. 2005; Koorem et al. 2011), liberar nutrientes (Facelli and Pickett 1991); o inmovilizarlos, debido a la liberación de compuestos alelopáticos (Foster y Gross 1998; Bonanomi et al. 2006; Samedani et al. 2013). Entre otros rasgos, la composición química de la hojarasca y su tasa de descomposición pueden tener un papel determinante sobre los efectos de la hojarasca en las plantas (Dorrepaal et al., 2007).

Los efectos de la hojarasca sobre las plantas suelen medirse en base a la biomasa aérea de las plantas (Dorrepaal et al. 2007; Fisher et al. 2013), aunque también se han estudiado sus efectos sobre la raíz (Bonanomi et al. 2011; Bughio et al. 2013). Sin embargo, no se han considerado algunos rasgos de la raíz, como la longitud específica (SRL) o el diámetro, que determinan su capacidad de exploración (Eissenstat 1992; Ostonen et al. 2007) o sus funciones (Eissenstat et al. 2000).

Nuestros resultados (capítulo 6) indican una gran variación de los efectos de la hojarasca sobre las plantas. Por ejemplo, la hojarasca de 5 especies de las 21 usadas en el estudio tuvieron efectos positivos (2 especies) y negativos (3 especies) sobre el crecimiento de la especie diana (Fig. 1). Las dos especies que provocaron un aumento en la biomasa total de la planta diana tenían una mayor concentración de Ca (Fig. 1). Es sabido que el Ca es capaz de modificar el pH de un suelo (Dauer 2007). Concretamente, nuestra especie diana (una herbácea, *D. glomerata*) ha demostrado un crecimiento mayor en respuesta a un incremento en los niveles de Ca en un sustrato arenoso (como el utilizado en nuestro ensayo; Crossley y Bradshaw 1968).

Por otro lado, encontramos una correlación negativa y consistente entre la biomasa y la longitud de la raíz de la especie diana y la concentración de polifenoles en la hojarasca (Fig. 1). Los polifenoles tienen un efecto inhibidor sobre la nitrificación, así como sobre la descomposición de la hojarasca (Verhoeven y Toth 1995) y el reciclado de nutrientes (Hättenschwiler y Vitousek 2000; Joanisse et al. 2007).

Por tanto, los resultados de nuestro estudio sugieren que el efecto positivo sobre el crecimiento de la especie diana, o, por el contrario, la inhibición del mismo, podrían ser el resultado de un balance entre la concentración de nutrientes y la de polifenoles en la hojarasca (Fig. 1). Así, el efecto inhibidor del crecimiento podría estar asociado a la liberación de compuestos alelopáticos (Bonanomi et al. 2006; Dorrepaal et al. 2007). Por otro lado, los posibles mecanismos que podrían explicar un crecimiento mayor con la hojarasca de determinadas especies podrían consistir en una baja concentración de polifenoles, sumada a un incremento en la disponibilidad de los nutrientes del suelo (Facelli y Pickett 1991).

Respecto al efecto de la hojarasca sobre la raíz, encontramos que aquellas especies que tenían hojarasca con una alta concentración de polifenoles, tenían una mayor proporción de raíces. Además, encontramos una fuerte correlación negativa entre la longitud específica de raíz (SRL) y la biomasa total. Es decir, las plantas más pequeñas (que eran las que tenían una hojarasca con mayor concentración de polifenoles), también tenían raíces más finas. Esto puede interpretarse como un cambio plástico en las raíces como respuesta a un efecto inhibitorio de la hojarasca sobre el desarrollo de la raíz y por tanto de la planta completa.

Respecto a los efectos de la hojarasca perteneciente a distintos grupos funcionales, encontramos que la hojarasca de perennifolias inhibía el crecimiento (Fig. 1). Esto puede deberse a lo comentado anteriormente referente a su composición química: menor concentración de Ca y mayor de polifenoles. En general, las especies leñosas perennifolias mediterráneas hacen una inversión mayor en la biosíntesis de compuestos secundarios basados en carbono (como los polifenoles) que en compuestos nitrogenados (Villar et al. 2006; Di Ferdinando et al. 2013). Se considera que algunos polifenoles intervienen en el engrosamiento de la pared celular secundaria (Di Ferdinando et al. 2013). En consecuencia, la hojarasca procedente de especies perennifolias se describe como estructuralmente dura y pobre en nitrógeno, y se descompone más lentamente que aquella de características opuestas (Gallardo y Merino 1993), reteniendo el N en la materia orgánica durante más tiempo, limitando así la producción primaria en ecosistemas con dominancia de estas especies (Terradas 2001).

En resumen, los rasgos funcionales pueden explicar algunos de los procesos y respuestas de las especies como son el crecimiento, la tolerancia a la sequía, la estructura y la composición química (sólo en el caso de las caducifolias) de las hojas senescentes y los efectos de la hojarasca sobre el crecimiento de otras especies. Conocer estas relaciones puede ser útil para dilucidar los posibles cambios de estos procesos y respuestas en un escenario de cambio global.

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Conclusiones generales

1. No existe una buena relación del crecimiento basado en incremento de altura con el basado en el incremento en diámetro. Esto subraya la importancia de realizar la estima del crecimiento de árboles adultos basándose en estas dos variables.
2. Existe una buena relación de las tasas de crecimiento relativo de las especies de *Quercus* en plántulas y adultos. Las tasas de crecimiento relativo, tanto en plántulas como en árboles adultos estaba positivamente relacionado con el área específica foliar y la concentración de nitrógeno.
3. Existe un compromiso entre el crecimiento y la supervivencia a la sequía en especies leñosas mediterráneas. Las especies adquisitivas, con una tasa de crecimiento rápido, tienen un área específica foliar elevada, raíces profundas pero baja área transpirante y por lo tanto, un cociente profundidad raíz/área foliar bajo, pero sobreviven poco tiempo a la sequía. Las especies conservativas tienen rasgos opuestos y sobreviven más tiempo a la sequía.
4. El tiempo de supervivencia a la sequía en condiciones controladas está muy relacionado con la distribución de las especies en el campo, aunque no es el único factor que la explica.
5. La sequía modifica la estructura y composición de las raíces: provoca un incremento en la longitud específica de la raíz (raíces más finas), en la densidad de los tejidos y una disminución en su diámetro.
6. Las especies con raíces más plásticas (con mayor capacidad para cambiar sus rasgos) tienen un mayor tiempo de supervivencia a la sequía.
7. Los rasgos estructurales de las hojas verdes tienen un papel determinante sobre los rasgos estructurales de la hojarasca.
8. La relación entre la concentración de nutrientes de la hoja verde y la senescente depende del grupo funcional. En caducifolias sí existe una fuerte relación entre la concentración de nutrientes de hoja verde y senescente mientras que en perennifolias esta relación no se cumple o es más débil.
9. Los grupos funcionales difieren únicamente en la reabsorción de P, que es mayor para perennifolias.

10. No se encontró ninguna relación entre los rasgos foliares y la eficiencia de reabsorción de carbono y nutrientes.
11. La adición de hojarasca al suelo tuvo efectos positivos, negativos o nulos sobre el crecimiento de otras especies, y éstos dependen en parte de su composición química, resultado del balance entre la concentración de nutrientes (efecto positivo) y polifenoles (efecto negativo).
12. La hojarasca de especies perennifolias tiene una mayor concentración de polifenoles y menor de Ca, por lo que inhibe el crecimiento, mientras que las hojas de las especies caducifolias tienen las características opuestas.

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Cuando empecé en esto era una niña. Yo no soy de esas personas que tienen todo definido. Todos los planes hechos, todo encauzado y cumplido según lo previsto. No. Para eso soy distinta. Y no crean ustedes que no cargo una gran cruz, que la llevo. Yo soy una persona llena de dudas. Siempre dudo de todo. De lo que hago, de lo que hacen, de lo que leo, de lo que dicen. Y precisamente el dudar tanto me ha traído hasta aquí. Fíjense que hasta dudaba de que algún día escribiría estas líneas. Así que ya ven, esta no es una historia de unos objetivos fijos y una superación de esos objetivos. Esta es la historia de una lucha. De una pelea entre lo que soy y lo que no soy. De lo que creo que puedo hacer y de lo lejos que puedo llegar. Y esta vez, miren qué curioso, me he llevado una sorpresa. Resulta que he perdido mi apuesta y aun así he salido ganando. Estos días me siento como si de repente tuviera superpoderes. Y, aunque sea más bien fruto del cansancio, está bien llevarse una alegría de vez en cuando, ¿no creen?

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Y como en la vida (igual que en ciencia) los resultados no son siempre positivos, para los resultados negativos está la familia. Estaría faltando la verdad si dijera que todos los momentos han sido buenos. Está bien quedarse con lo positivo, pero es aún más importante quedarse con aquellos que te apoyan y acompañan en lo negativo. Y esa ha sido mi familia, la verdadera protagonista de esta sección. Escuché a alguien decir un día: el verdadero amor es aquél que te lleva al médico cuando estás enfermo. Y esa es una gran verdad.

Cada día y cada experiencia por la que paso, soy más consciente de la suerte que tuve cuando la cigüeña me dejó en esta casa y no en otra. Mi familia es lo mejor que tengo, y de lo que más orgullosa estoy. La familia para mí es TODO.

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‘Y aunque yo no te merezca,
Torpe, lacio e indeciso
Yo soy el guapo que pesca
Atunes en el paraíso.

No seré lo que querías,
Pero soy quien más te quiso
No se pescan to´los días
Atunes en el paraíso

Ya no hay nada que me importe
Yo cumplí mi compromiso
No se pescan por deporte
Atunes en el paraíso’

Javier Ruibal