Universidad
de Alcalá

Revegetación de campos agrícolas abandonados en ambientes mediterráneos continentales

De la ecofisiología de los individuos a las propiedades ecosistémicas

Tesis doctoral

Bárbara Cuesta Poveda

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Memoria presentada por Dña. **Bárbara Cuesta Poveda**
para optar al grado de Doctora por la Universidad de Alcalá

Directores
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Alcalá de Henares, a 8 de febrero de dos mil diez.

Dr. Julio Camargo Benjumeda
Director del Departamento

A mis padres,
y hermanos

Una experiencia que acaba
no sólo abre una ventana a un mundo de posibilidades
sino que refleja todo lo aprendido en el camino,
las lágrimas vertidas y la felicidad gestada.
Tiempo de vida dando lo mejor de uno mismo.

Virginia Sanz Pérez

Resumen

Esta Tesis aborda un conjunto de procesos y técnicas para comprender y mejorar, respectivamente, la revegetación de campos agrícolas abandonados en ambientes mediterráneos continentales. Los objetivos específicos fueron: a) estudiar los mecanismos que explican por qué los plantones de gran tamaño y concentración de nitrógeno (N) (fenotipos *productivos*) se establecen mejor que los plantones con los atributos contrarios (fenotipos *xeromorfos*) en repoblaciones forestales en ambientes mediterráneos ; b) analizar la interacción entre los atributos funcionales de los plantones con el nivel de estrés del ambiente de plantación; c) evaluar la utilidad de *Retama sphaerocarpa* como arbusto nodriza en proyectos de revegetación, analizando la facilitación tanto directa como indirecta sobre plantones de *Quercus ilex*; d) conocer cómo la interrupción del manejo aplicado durante la fase de establecimiento influye en el funcionamiento futuro de los individuos de *Q. ilex*; y e) conocer los efectos que la revegetación tiene en las propiedades químicas del suelo.

Para ello se llevaron a cabo varios experimentos y un estudio observacional en el centro de España en los que se examinó 1) la supervivencia, crecimiento y fisiología de plantones de *Q. ilex* y *Pinus halepensis* Mill. de tamaño y estado nutricional contrastados plantados en dos ambientes de diferente estrés hídrico; 2) el efecto del tamaño de la planta y de la concentración de N en el desarrollo radical y su interferencia con la elongación aérea en plantones de *P. halepensis*; 3) el efecto que *R. sphaerocarpa* y la vegetación herbácea tienen en la supervivencia, crecimiento y respuesta fisiológica de plantones de *Q. ilex*; 4) la supervivencia, crecimiento y producción de semillas en individuos de *Q. ilex* de 14 años de edad, comparando los efectos directos del manejo previo (sombra artificial y riego estival) con los efectos indirectos a través de la competencia intraespecífica y los compromisos del reparto de recursos entre distintas funciones básicas de las plantas; y 5) la variación en el pH, concentración de macronutrientes y la tasa de nitrificación y amonificación en el suelo en escenarios contrastados de restauración activa y pasiva de la vegetación.

Los plantones de *P. halepensis* grandes y con gran concentración de N presentaron mayor supervivencia y crecimiento que los plantones pequeños, independientemente de la concentración de N en sus tejidos. Ello se asocia con un mayor crecimiento radical, intercambio gaseoso y uso del N en los plantones grandes que en los pequeños. El tamaño del plantón tuvo un efecto mayor en su establecimiento en el campo que la concentración de N en *P. halepensis*. El crecimiento radical interfirió el crecimiento aéreo en *P. halepensis*, pero este efecto sólo se observó en los plantones más pequeños. En *Q. ilex* no se observaron diferencias de establecimiento y funcionamiento entre tipos de planta o éstas fueron muy pequeñas .

Retama sphaerocarpa facilitó a *Q. ilex*, variando la importancia relativa del mecanismo de facilitación con las condiciones climáticas de los años estudiados. La facilitación fue indirecta, mediante la reducción de la capacidad competitiva de la comunidad herbácea, en el año de primavera seca. En cambio, la facilitación fue predominantemente directa, mediante la reducción de la radiación y la temperatura bajo su dosel, en el año de primavera húmeda y verano seco. No todos los individuos de *Q. ilex* respondieron de la misma forma a la facilitación ya que benefició a los plantones pequeños pero no a los grandes.

El crecimiento y la producción de bellotas en el presente de individuos de *Q. ilex* de 14 años de edad fue, en parte, determinado por el ambiente pasado (manejo inicial), tanto por el efecto directo del manejo como por los efectos indirectos a través de la competencia intraespecífica y de la reproducción de los individuos. No se observaron compromisos en la asignación de recursos entre el crecimiento aéreo y el radical, ni entre el crecimiento y la supervivencia, pero sí entre el crecimiento y la producción de bellotas, aunque esto sólo ocurrió en los individuos relativamente grandes cuyo dosel era mayor de 2 m^3. El efecto de la plantación de estos individuos y de la restauración pasiva en las propiedades químicas del suelo después de 13 años fue muy pequeño. La concentración de amonio y la disponibilidad de N mineral en el suelo fue mayor en las parcelas restauradas de forma activa que en las parcelas restauradas de forma pasiva.

Los resultados obtenidos en esta Tesis Doctoral mejoran nuestra compresión de los procesos subyacentes al establecimiento de árboles en un proyecto de revegetación, lo que puede ser de gran importancia en la restauración de tierras agrícolas abandonadas en paisajes mediterráneos y otras regiones secas del planeta.

Palabras clave: calidad de planta, competencia, compromisos en la asignación de recursos, concentración de nitrógeno, crecimiento de raíces, cultivo en vivero, efectos del ambiente previo, facilitación, intercambio gaseoso, potencial hídrico, removilización, repoblación, tamaño del plantón, propiedades edáficas.

Abstract

This PhD thesis investigates processes and techniques to understand and improve, respectively, the revegetation of abandoned croplands in Mediterranean continental environments. Specific aims were: a) to study the mechanisms that explain why large and high nitrogen (N) concentration seedlings (*productive* phenotypes) establish better than seedlings with the opposite traits (*xeromorphic* phenotypes) in Mediterranean reforestations; b) to analyse the interaction between seedling functional traits and environmental stress; c) to evaluate the use of *Retama sphaerocarpa* as nurse shrub in revegetation projects, analysing both direct and indirect facilitation of *Quercus ilex* seedlings; d) to examine how the interruption of the management applied during seedling establishment affects future performance of *Q. ilex* individuals; and e) to identify the effects of revegetation on soil chemical properties.

We carried out four experiments and one observational study in the centre of Spain to examine: 1) survival, growth and physiological response of *Q. ilex* and *Pinus halepensis* Mill. seedlings of different size and N concentration planted in two contrasting water stressed environments; 2) effects of seedling size and N concentration on root growth and its interference with aerial elongation in *P. halepensis* seedlings; 3) *R. sphaerocarpa* and herb effects on survival, growth and physiological response of *Q. ilex* seedlings; 4) survival, growth and acorn production in a 14 year-old *Q. ilex* individuals, to compare direct effects of previous management (artificial shading and summer irrigation) and indirect effects through intraespecific competition and resource allocation to plant basic functions; and 5) soil variation in pH, macronutrient concentration, and nitrification and ammonification rates in contrasting scenarios of passive and active restoration.

Large seedlings of *P. halepensis* with high N concentration had higher survival and growth than small seedlings, independently of tissue N concentration. This was attributed to higher root growth, gas exchange and N use in large seedlings compared to small seedlings. Size effect on seedling establishment was higher than N concentration effect. Root and shoot growth interfered in *P. halepensis*, but only in smaller seedlings. In *Q. ilex*, there were no establishment and physiological differences among seedling phenotypes or they were marginal .

Retama sphaerocarpa facilitated *Q. ilex*, but the relative importance of the facilitation mechanism varied with the climatic conditions of the studied years. Indirect facilitation by release of herb competition under nurse shrubs occurred in the year with dry spring. By contrast, direct facilitation mediated by microclimate amelioration occurred in the year with humid spring and dry summer. *Q. ilex* seedlings responded differently to facilitation, since small seedlings but not large seedlings were benefited.

Current growth and acorn production in 14 year-old *Q. ilex* individuals was, in part, determined by previous environment (initial management), due to both direct effects of management and indirect effects through intraespecific competition and acorn production. There were no trade-offs in resource allocation either between root and shoot growth or between growth and survival, but there was a trade-off between growth and acorn production that was only noticeable in individuals with canopy higher than 2 m^3. Active restoration through *Q. ilex* plantation and passive restoration had low and similar effects on soil chemical properties after 13 years. Actively restored plots had higher ammonium concentration and mineral N availability than passively restored plots.

The results of this PhD thesis improve our understanding of processes related to tree establishment in revegetation projects, which can be important for abandoned cropland restoration in Mediterranean environments and other dry systems of the world.

Key words: competition, effects of previous environment, facilitation, gas exchange, nitrogen concentration, nursery cultivation, plant quality, remobilisation, reforestation, resource allocation trade-offs, root growth, seedling size, soil properties, water potential.

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Sugus entre cuatro

Aprendí...
que es más dar que tener,
que es más ser que hacer,
que es más vivir y llorar,
que andar sin mirar.
Que es más amar que hablar,
sentir que decir...
esto al vagar,
lo aprendí de tí.

Luis Merino Martín

Capítulo 1

Capítulo 1

Introducción general

Durante las últimas décadas se han abandonando en el mundo grandes superficies de tierras agrícolas debido a factores ecológicos (sobreexplotación agrícola y ganadera, relieve pronunciado, clima, erosión, etc.) y socioeconómicos (baja productividad de los cultivos, cambios sociales, políticas agrarias, etc.) (Aide y Grau 2005; Mottet *et al*. 2006; Rey Benayas *et al*. 2007). Tras su abandono, estas superficies pueden ser objeto de la sucesión secundaria o restauración pasiva de la vegetación (Debussche *et al*. 1996), o pueden ser restauradas de forma activa, es decir, pueden ser manejadas para el reestablecimiento del ecosistema original (SER 2004). La restauración pasiva ha restaurado en el mundo mucha más superficie y a un coste menor que la restauración activa (Rey Benayas 2005). Sin embargo, en ambientes poco productivos el proceso puede ser muy lento o desembocar en terrenos aún más degradados. Así, la restauración activa es necesaria en zonas que sufren degradación continua, donde la cubierta vegetal no se puede recuperar por sí sola, cuando es deseable acelerar la sucesión secundaria, o se persiguen beneficios más allá de la revegetación *per se* como puede ser la creación de empleo y renta.

El reclutamiento de especies leñosas en ambientes mediterráneos se ve limitado por la elevada temperatura y sequía estival, la baja disponibilidad de nutrientes y perturbaciones recurrentes características de estos ambientes tales como la erosión, el fuego y la herbivoría (Gallardo 2001; Castro-Díez 2003; Castro *et al*. 2004; Lloret 2004). Los campos agrícolas abandonados se caracterizan, además, por la presencia de un gran banco de semillas de especies herbáceas en el suelo que, junto con los trabajos de laboreo realizados anteriormente, favorece la colonización de especies ruderales, las cuales ejercen una fuerte competencia por los recursos edáficos, particularmente el agua (Rey Benayas *et al*. 2002; Rey Benayas *et al*. 2005). Todos estos factores ralentizan la regeneración natural de la vegetación leñosa mediterránea, por lo que la restauración activa es necesaria para transformar campos agrícolas en ecosistemas forestales o agroforestales en pocos años.

Los objetivos de la restauración ecológica pueden ser múltiples, dependiendo del tipo de ecosistema a restaurar, de su grado de degradación y de las limitaciones ecológicas y socioeconómicas. No obstante, Vallejo *et al*. (2006) sugieren que los objetivos básicos de cualquier proyecto de restauración ecológica en la Cuenca Mediterránea deben ser 1) detener la degradación, 2) mejorar la función y estructura a nivel tanto de ecosistema como de paisaje, 3) impulsar la sucesión secundaria estimulando la regeneración natural, 4) incrementar la resiliencia del ecosistema a perturbaciones como el fuego, las sequías extremas y el calentamiento global y 5) promocionar sistemas naturales y culturales autosuficientes, sostenibles y sanos. La recuperación de las propiedades y procesos ecosistémicos a menudo requiere el reestablecimiento previo de la cubierta vegetal (Young 2000; Jansen 2005; Vallejo *et al*. 2006; Marcos *et al*. 2007; Smal y Olszewska 2008; Rebecca *et al*. 2009), lo que hace que la revegetación sea necesaria para la restauración ecológica de campos agrícolas abandonados en los que la recolonización natural de la vegetación forestal madura es muy lenta en relación a las escalas de tiempo de las sociedades humanas.

En la Península Ibérica, la mayor parte de los trabajos de revegetación con especies leñosas que se han llevado a cabo han sido repoblaciones forestales, las cuales se han limitado a la introducción de especies arbóreas (Cuadro 1). Las especies más utilizadas han sido de crecimiento rápido, como los pinos (*Pinus halepensis*, *P. pinaster*, etc.) y el eucalipto (*Eucaliptus* spp.), ya que proporcionan una rápida recuperación de la cubierta y presentan menos problemas de establecimiento que otras especies (MAPA 2006). Durante la última década, sin embargo, se ha incrementado el uso de otras especies, tanto arbustivas (*Retama sphaerocarpa*, *Pistacia lentiscus* y *Quercus coccifera*, entre otras) como arbóreas (*Quercus ilex* y *Quercus suber*, entre otras) (MAPA 2006). La evidencia empírica apunta a que en algunos casos, como en las especies del género *Quercus*, la supervivencia de las plantaciones de frondosas nativas es baja o muy baja. La puesta a punto de técnicas que faciliten la introducción de estas especies debería ser, por tanto, un objetivo prioritario para mejorar el éxito de los proyectos de revegetación o repoblación. En este aspecto se han hecho avances significativos (Rey Benayas 1998; Barberá *et al*. 2005; Naverro Cerrillo *et al*. 2005; Jímenez *et al*. 2007; entre otras).

Aspectos importantes en un proyecto de restauración de la vegetación

Un buen proyecto de restauración de la vegetación forestal debe considerar al menos los siguientes aspectos: 1) una correcta elección de la planta que va a ser introducida (Duryea 1985; Villar-Salvador 2003; Oliet *et al*. 2009a), 2) las prácticas culturales que faciliten la implantación de las especies seleccionadas (Rey Benayas 1998; Navarro Cerrillo *et al*. 2005; Jiménez *et al*. 2007), y 3) el seguimiento y la evaluación de la revegetación para cuantificar el éxito del proyecto y conocer cuál ha sido su efecto en la restauración del ecosistema (Ruiz-Jaen y Aide 2005; Rey Benayas *et al*. 2008).

Importancia de las características funcionales de la planta introducida

En un proyecto de revegetación es importante elegir las especies más adecuadas para la zona a revegetar según las limitaciones ecológicas del terreno y los objetivos de la restauración. También, dentro de cada especie seleccionada, es necesario elegir plantones con las características funcionales apropiadas para que la plantación tenga éxito, es decir, plantones de calidad. Duryea (1985) define una planta de calidad como aquella que es capaz de alcanzar un desarrollo (supervivencia y crecimiento) óptimo en un medio determinado y, por tanto, cumplir los objetivos establecidos en un plan de restauración. No existe un único modelo de calidad de planta sino que depende de las especies, características del ambiente y los objetivos de la restauración con los que se trabaje (Villar-Salvador 2003; Cortina *et al*. 2006).

Cuadro 1

La repoblación forestal en España

Las primeras repoblaciones forestales en España datan de la Baja Edad Media, pero es a finales del siglo XIX cuando éstas afectan a grandes superficies de terreno, generalmente utilizando plantaciones monoespecíficas de pinos (Valdés y Gil 1998; MAPA 2006). Tras la Guerra Civil, la actividad repobladora aumentó de forma considerable, ya que el régimen franquista consideraba esta actividad como dinamizadora de la economía en zonas rurales (MAPA 2006). Además, las sucesivas inundaciones en distintas partes del territorio español debido a la gran deforestación de las cuencas fluviales hicieron que se repoblaran grandes superficies con fines de restauración hidrológica. Durante estos años, se procedió al uso masivo de especies de crecimiento rápido como pinos y eucaliptos, que presentaban un fácil manejo en vivero, un mercado favorable (madera, pasta de papel, resina) y tardaban menos tiempo en alcanzar la espesura completa (Vallejo *et al*. 2003). En la segunda mitad de la década de los 80 del siglo XX las competencias en la gestión forestal pasaron a las comunidades autónomas, que redujeron la actividad repobladora notablemente (Gómez Orea 2004).

Figura 1. Repoblación de *Pinus pinaster* realizada en los años 70 en la provincia de Guadalajara.

En 1992, la reforma de la Política Agraria Comunitaria (PAC) impulsó de nuevo las plantaciones forestales al incentivarse la reconversión de terrenos agrícolas en terrenos forestales, si bien los objetivos fueron, principalmente, eliminar excedentes agrícolas y proporcionar renta a una población rural cada vez menos numerosa y envejecida, y no los objetivos ambientales asociados. Desde la entrada en vigor de esta reforma en 1994 hasta el año 2006 se repoblaron 685.000 ha de tierras agrícolas (MAPA 2006). Entre las especies más utilizadas destacan *Pinus halepensis* y *Quercus ilex*, si bien se ha impulsado la introducción de otras especies, mezclando frondosas con coníferas, y especies arbustivas con arbóreas en el mismo proyecto (MAPA 2006).

Tradicionalmente se ha considerado que a nivel intraespecífico los plantones de pequeño porte, con una relación entre el tamaño de la parte aérea y el sistema radical reducida y bajo contenido de nutrientes (que en esta Tesis denominaremos fenotipos *xeromorfos*), son los más adecuados para sobrevivir en ambientes mediterráneos, ya que consumen menos agua que las plantas de atributos contrarios, es decir, plantas de gran porte con una relación entre el tamaño de la parte aérea y el sistema radical elevada y elevado contenidos de nutrientes (que denominaremos fenotipos *productivos*) (Thompson 1985; Leiva y Fernández-Alés 1998). En ambientes muy secos se ha visto que las plantas de fenotipo *xeromorfo* pueden desarrollarse mejor que las de fenotipo *productivo* (Rose *et al*. 1993; Trubat *et al.* 2008). No obstante, en los últimos años han aparecido numerosos estudios que demuestran que cuando existe una relación entre el establecimiento en campo y los atributos funcionales de la planta, los fenotipos *productivos* pueden desarrollarse mejor que los *xeromorfos* tanto en ambientes secos (Puértolas *et al*. 2003; Tsakaldimi *et al*. 2005; Villar-Salvador *et al*. 2008)como incluso en los semiáridos (Luis *et al*. 2009; Oliet *et al*. 2009a). Resultados similares se han obtenido en ambientes mésicos, donde la plantación de brinzales de gran tamaño supone una alternativa al uso de herbicidas en sitios de competencia herbácea intensa, dada su mayor capacidad competitiva en comparación con plantones de menor tamaño (Lamhamedi *et al*. 1998; Noland *et al*. 2001; South *et al*. 2005). Siguiendo esta línea, en ambientes mediterráneos se está empezando a utilizar brinzales de gran porte en obras civiles y en determinados proyectos agroforestales.

El estado nutricional de la planta también puede condicionar la capacidad de desarrollo post-plantación (Malik y Timmer 1996; Salifu y Timmer 2003). Se ha observado que las plantas muy fertilizadas y, por tanto, con concentración y contenido de nutrientes elevados en los tejidos presentan mayores tasas de supervivencia y crecimiento que las plantas poco fertilizadas y, por tanto, con un estado nutricional pobre (van den Driessche 1988; Malik y Timmer 1996; Puértolas *et al*. 2003; Salifu y Timmer 2003). Una elevada concentración de nitrógeno (N) puede traducirse en mayores tasas fotosintéticas (Jose *et al*. 2003; Gough y Seiler 2004). Además, un mayor contenido de N permite una mayor removilización de este nutriente para apoyar el crecimiento de los nuevos órganos (Millard 1996; Silla y Escudero 2003; Oliet *et al*. 2009b). No obstante, un exceso de nutrientes puede ser perjudicial para las plantas (Oliet *et al*. 2006). Algunos trabajos sugieren que concentraciones elevadas de N en plantas cultivadas en contenedor producen un desequilibrio entre la parte aérea y la radical, dificultando su balance hídrico y su supervivencia (Tan y Hogan 1997; Trubat *et al*. 2008). A veces también limita la capacidad de aclimatación fisiológica al frío y a la sequía (Tan y Hogan 1997; Fløistad y Kohmann 2004).

A pesar de la frecuente relación positiva existente entre el desarrollo en campo de los plantones con su tamaño y estado nutricional el conocimiento de los mecanismos ecofisiológicos subyacentes en estas relaciones es escaso. En este sentido, Villar-Salvador (2003) sugirió que el establecimiento de una planta depende del grado de desarrollo del sistema radical antes del periodo estival. Aquellas características funcionales que favorezcan el potencial productivo de la planta (mayor follaje y contenido de nutrientes y alta tasa de fotosíntesis, entre otras) redundarán en sistemas radicales más extensos y, en consecuencia, en una mayor probabilidad de supervivencia y capacidad de crecimiento. Un modelo similar ha sido propuesto por Luis *et al*. (2009) para explicar el establecimiento de *Pinus canariensis*.

Una buena parte de los estudios que analizan la relación existente entre el desarrollo post-transplante de las plantas y sus características funcionales provienen de ensayos de fertilización. La fertilización durante la fase de cultivo en vivero afecta tanto al tamaño como al estado nutricional de los plantones (van den Driessche 1988; Villar-Salvador *et al.* 2008; Oliet *et al*. 2009a). Al recibir mayor fertilización las plantan crecen más, ya que disponen de más nutrientes para hacerlo, pero también almacenan más reservas, incrementando así la concentración de nutrientes en sus tejidos. Dado que la fertilización en vivero afecta simultáneamente al tamaño y a la concentración de nutrientes de los plantones, no es posible diferenciar cuál es la contribución específica de cada uno de estos atributos en el establecimiento en campo de los plantones. Son, por tanto, necesarios experimentos que analicen cuál es el peso específico de la morfología y la nutrición en la respuesta post-transplante de los plantones.

Compromisos en la asignación de recursos

La disponibilidad de recursos que las plantas necesitan para sobrevivir es a menudo limitada, por lo que no pueden atender eficazmente a la vez todas sus funciones básicas (crecimiento, mantenimiento, metabolismo secundario y reproducción). Invertir recursos en una función supone detraer recursos de las demás, lo que implica un coste para la planta y da lugar a compromisos de asignación de recursos entre los distintos componentes del ciclo vital o rasgos de vida: crecimiento, reproducción y supervivencia (Bazzaz *et al*. 1987; Reekie y Bazzaz 1987; Ashman 1994). A pesar de que se considera un principio establecido que las distintas funciones de las plantas compiten por los mismos recursos, no todas las investigaciones han dado los mismos resultados, poniendo en duda que la existencia de compromisos entre los distintos rasgos de vida sea una pauta universal (Fox 1995; Despland y Houle 1997; Antos y Allen 1999).

Además, dado que los recursos en el medio son limitados, las plantas se ven obligadas a compartirlos, lo que da lugar a fenómenos de competencia entre los individuos. Algunos autores han demostrado que los fenómenos de competencia pueden desencadenar cambios en los patrones de asignación de recursos. En escenarios de competencia por la luz, las plantas suelen invertir más recursos en el crecimiento de la parte aérea para una mayor captación de luz, y aumentan la asignación de recursos a los tallos a expensas de hacerlo a hojas y raíces (Nilsson y Hallgren 1993; Maliakal *et al*. 1999; Watt *et al*. 2003). Cuando la competencia es por los recursos del suelo, las plantas aumentan la inversión en el sistema radical y disminuye el crecimiento de la parte aérea (Giordano y Hibbs 1993). Los compromisos de asignación de recursos entre órganos o funciones pueden, por tanto, variar según las condiciones ambientales en las que vive la planta. Es probable, así mismo, que estos patrones de asignación varíen según los recursos almacenados en la planta.

Prácticas culturales en la restauración de la vegetación

Las numerosas marras existentes en los proyectos de revegetación o repoblación forestal de ambientes mediterráneos (Castro *et al*. 2004; Navarro Cerrillo *et al*. 2006), hacen necesaria la aplicación de técnicas culturales que incrementen la supervivencia de los plantones y aseguren el éxito de las plantación como la provisión de sombra y el riego (Rey Benayas 1998; Bainbridge 2002; Jiménez *et al*. 2007), la disminución de la competencia con las hierbas (Navarro Cerrillo *et al*. 2005; Rey Benayas *et al.* 2005) y la preparación del suelo (Querejeta *et al*. 2001), entre otras. Estas prácticas, sin embargo, suelen elevar el coste de la revegetación, por lo que es necesaria la búsqueda de alternativas que garanticen la supervivencia de la plantación a bajo costo y reducido impacto ambiental.

En la naturaleza las plantas interaccionan entre sí, ejerciendo sobre sus vecinos tanto efectos positivos como negativos (Maestre *et al*. 2009). Cuando el resultado de este balance es positivo, una planta favorece el establecimiento de otras que crecen próximas a ella, lo que se denomina facilitación o efecto nodriza (Callaway 1995). Varios autores proponen utilizar este efecto nodriza en la restauración de la vegetación (Gómez-Aparicio *et al*. 2004; Padilla y Pugnaire 2006) (Cuadro 2).

La facilitación puede ser directa, cuando una planta genera cambios ambientales *sensu lato* bajo su dosel (mejora en las condiciones microclimáticas y/o edáficas) que favorecen el desarrollo de otra planta (Callaway 1995; Pugnaire *et al*. 1996a; Pugnaire *et al*. 2004), o indirecta, cuando interactúan tres o más organismos y como resultado uno de ellos se ve beneficiado (protección frente a herbívoros, atracción de polinizadores o supresión de un competidor común) (Miller 1994; Levine 1999; Callaway 2007). El papel que tiene la facilitación directa en el reclutamiento de especies leñosas ha sido muy estudiado (Callaway 1992; Barnes y Archer 1999; Kitzberger *et al*. 2000; Maestre *et al*. 2001; Oakley *et al*. 2006). Sin embargo, el papel de la facilitación indirecta ha recibido menos atención. Dentro de los estudios que abordan la facilitación indirecta la mayoría se centran en la protección frente a la herbivoría (García y Obeso 2003; Boulant *et al*. 2008; Gómez-Aparicio *et al.* 2008) o a la atracción de polinizadores y/o dispersadores de propágulos (Verdú y García-Fayos 2003; Aerts *et al.* 2006), mientras que los estudios que demuestran facilitación indirecta en un mismo nivel trófico son muy escasos (Li y Wilson 1998; Siemann y Rogers 2003; Kunstler *et al*. 2006).

Los procesos de facilitación directa han sido muy documentados en matorrales mediterráneos (Castro *et al*. 2004; Gómez-Aparicio *et al*. 2004; Castro *et al*. 2006; Smith *et al*. 2008). Sin embago, hasta donde conocemos, ningún estudio ha abordado la existencia de facilitación indirecta entre plantas. Una de las especies nodriza mediterránea más estudiada es *Retama sphaerocarpa* (retama), un arbusto que promueve bajo su copa el desarrollo de una abundante y diversa comunidad herbácea (Pugnaire *et al*. 1996b; Moro *et al.* 1997; Rodríguez-Echeverría y Pérez-Fernández 2003; Pugnaire *et al*. 2004) y de otras especies leñosas de etapas medias y tardías en la sucesión (Tovar 2009). Muchos trabajos han demostrado la existencia de facilitación directa bajo retama (Pugnaire *et al.* 1996b; Moro *et al*. 1997; Rodríguez-Echeverría y Pérez-Fernández 2003), mientras que ninguno ha demostrado la posible existencia de facilitación indirecta.

El resultado de la interación entre dos organismos (facilitación o competencia) depende de las especies consideradas (Gómez-Aparicio *et al*. 2004; Liancourt *et al*. 2005; Padilla y Pugnaire 2009). Sin embargo, dentro de una misma especie, los individuos poseen distintas características funcionales, lo que puede determinar su establecimiento tal como se explicó anteriormente. Así, por ejemplo, las plántulas relativamente grandes, como las crecidas a partir de semillas grandes, tienen más posibilidades de sobrevivir que las plántulas pequeñas cuando crecen en un ambiente estresante como el provocado por la competencia con hierbas o el estrés hídrico y lumínico (Cook 1980; Leishman y Westoby 1994). Hasta el momento ningún trabajo ha analizado si a escala intraespecífica el resultado de la interación entre plantas depende de las características funcionales de los individuos facilitados.

Cuadro 2.

El uso de plantas nodrizas como técnica de revegetación

Tradicionalmente, antes de reforestar una zona se eliminaba la vegetación existente para evitar la posible competencia entre ésta y los plantones que iban a ser introducidos. En los últimos años, sin embargo, se ha observado que en algunos casos, especialmente en ambientes poco productivos, la vegetación existente facilita el establecimiento de plántulas y plantones leñosos gracias al micro-ambiente favorable que genera bajo su dosel (Fig. 2): menor desecación del suelo (Pugnaire *et al.* 2004; Gómez-Aparicio *et al*. 2008), disminución de la irradiación (Franco y Nobel 1989), menor temperatura del aire y del suelo (Castro *et al*. 2002; Gómez-Aparicio *et al*. 2005), mayor disponibilidad de nutrientes en el suelo (Pugnaire *et al.* 1996a; Moro *et al*. 1997) y mayor protección frente a los herbívoros (Boulant *et al*. 2008), principalmente. Este fenómeno se ha observado sobre todo bajo la copa de arbustos (Castro *et al*. 2004; Gómez-Aparicio *et al*. 2004; Smith *et al*. 2008; Gómez-Aparicio 2009), pero también bajo la de árboles (Siemann y Rogers 2003; Maestre *et al*. 2004) e incluso herbáceas (Maestre *et al*. 2001). En este contexto, algunos autores sugieren emplear la facilitación entre plantas para la introducción de especies leñosas forestales en los proyectos de revegetación (Castro *et al*. 2004; Gómez-Aparicio *et al*. 2004; Padilla y Pugnaire 2006).

Las interacciones positivas entre plantas son más frecuentes en ambientes sometidos a gran estrés abiótico (Bertness y Callaway 1994, ver discusión en Maestre *et al*. 2005, Lortie y Callaway 2006 y Maestre *et al*. 2006), por lo que es de esperar que la aplicación de la facilitación como técnica de revegetación sea más exitosa en ambientes estresantes, por ejemplo, en ecosistemas áridos o semiáridos, que en ambientes muy productivos, como humedales o bosques húmedos templados (Gómez-Aparicio 2009). En estos últimos, las plantas compiten fuertemente por la adquisición de los recursos esenciales (luz, agua y nutrientes) y la cercanía espacial de los vecinos tiene generalmente un resultado neto negativo (Choler *et al*. 2001; Pagès *et al*. 2003).

La utilidad del uso de plantas nodrizas en la restauración de la vegetación ha sido demostrado en múltiples ambientes: montañas mediterráneas (Castro *et al.* 2004; Gómez-Aparicio *et al*. 2004), estepas semiáridas (Maestre *et al*. 2001; Gasque y Garcia-Fayos 2004), bosque seco tropical (Aerts *et al*. 2006), bosque tropical húmedo (Ashton *et al*. 1997), pastizales (Huber-Sannwald y Pyke 2005; King y Stanton 2008) y campos abandonados en ambientes semiáridos (Padilla y Pugnaire 2009).

Figura 2. Individuo de *Juniperus oxycedrus* reclutado de forma natural bajo el dosel de *Retama sphaerocarpa* en un retamar mediterráneo.

Seguimiento y evaluación de la restauración de la vegetación

El seguimiento y evaluación de un proyecto de revegetación permite medir su éxito e identificar las acciones correctivas necesarias a medio y largo plazo (Vallauri *et al*. 2005). Además, permite conocer su efecto en la restauración del ecosistema. La mayoría de los trabajos evalúan el éxito de la restauración según tres grupos de características ecosistémicas: 1) la estructura de la vegetación, por ejemplo el porcentaje de la cubierta vegetal alcanzada, densidad, biomasa, etc. (Cooper y MacDonald 2000; Ward *et al*. 2000; Young 2000); 2) la diversidad, por ejemplo la riqueza y abundancia de organismos, tanto de plantas como de animales (Longcore 2003; Martin *et al*. 2005; Cayuela *et al*. 2008; Ndour *et al*. 2008); y 3) los procesos ecológicos, como son las interacciones biológicas, el ciclo de nutrientes, el secuestro de carbono y la regulación hidrológica (Harden y Mathews 2000; Silver *et al*. 2000; Meyer y Sisk 2001; Smal y Olszewska 2008). La recuperación de la estructura de la vegetación o de la diversidad es valorada en la gran mayoría de trabajos de restauración, ya que su medición es rápida y sencilla y se considera un paso necesario para la posterior recuperación de ciertas propiedades y procesos ecosistémicos (Young 2000; Ruiz-Jaen y Aide 2005). Por el contrario, el seguimiento del reestablecimiento de procesos ecosistémicos es menos frecuente debido principalmente a que es un proceso más lento (Kindscher y Tieszen 1998; Camill *et al.* 2004) y a que su evaluación requiere múltiples medidas, lo que incrementa el coste y duración del proyecto de restauración (Ruiz-Jaen y Aide 2005).

Seguimiento de la revegetación: respuesta a la interrupción de las prácticas culturales

Las prácticas culturales que facilitan el establecimiento de los plantones (riego, sombra, enmiendas orgánicas, control de la vegetación herbácea, etc.) son costosas, por lo que generalmente se aplican durante un periodo de tiempo reducido hasta garantizar el establecimiento de las plantas. Tras la interrupción del manejo inicial, los plantones se enfrentan a un ambiente muy diferente al existente durante su establecimiento. De ahí que sea importante evaluar su respuesta una vez que los cuidados cesan, ya que la respuesta presente de los organismos está influenciada no sólo por las condiciones ambientales actuales sino también por las condiciones ambientales previas (Metcalfe y Monaghan 2001; Relyea 2002; Cayuela *et al*. 2008). La forma en que las plantas reparten los recursos entre sus diferentes funciones (crecimiento, mantenimiento, metabolismo secundario y reproducción) está determinada genéticamente, pero son capaces de modificarla para optimizar la captación de recursos según su disponibilidad y las condiciones ambientales en que se encuentren (Bloom *et al*. 1985).

Evaluación del impacto de la revegetación en las propiedades ecosistémicas: cambios en las propiedades químicas del suelo

La agricultura influye de forma significativa en las propiedades químicas del suelo, aumentando el pH, disminuyendo la concentración de carbono e incrementando la concentración de fósforo y potasio, entre otras muchas (Koerner *et al*. 1997; Compton y Boone 2000). Estudios previos han demostrado que la revegetación con especies leñosas forestales de campos agrícolas incrementa el secuestro de carbono y de nutrientes en la biomasa aérea (Garten 2002; Uri *et al*. 2007). Sin embargo, su efecto en la acumulación de carbono (Garten 2002; Vesterdal et al. 2002; Smal y Olszewska 2008) y nutrientes (Ritter *et al*. 2003; Wall y Hytonen 2005; Falkengren-Grerup *et al.* 2006) en el suelo no es tan claro. Éstos y otros trabajos indican que la cantidad de nutrientes en el suelo y sus propiedades químicas dependen no sólo del uso previo del suelo, sino también de las condiciones ambientales, la microfauna edáfica y la vegetación asociada (Aerts y Chapin 2000; Paul *et al.* 2003).

Dentro de una misma región climática, la cantidad y calidad de la hojarasca juega un papel fundamental en la descomposición de la materia orgánica y el ciclo de nutrientes (Vinton y Burke 1995; Cornelissen *et al.* 1999). Así, por ejemplo, Gallardo y Merino (1993) demostraron que en ambientes mediterráneos la tasa de descomposición de la hojarasca depende de su contenido en carbono, lignina y cutina. Algunas características funcionales de las plantas como son su patrón de asignación de biomasa, la composición química de sus tejidos o su ciclo de vida pueden, por tanto, influir de forma significativa en las propiedades químicas del suelo (Hooper y Vitousek 1998; Carrera *et al*. 2009).

En ambientes mediterráneos, los campos agrícolas sujetos a sucesión son inicialmente colonizados por una abundante y diversa comunidad herbácea que persiste durante años hasta que, si es posible, se establecen especies leñosas (Bonet y Pausas 2004), mientras que en parcelas restauradas de forma activa la introducción de especies leñosas es directa (Fig. 3). Dado que el tipo de cubierta vegetal juega un papel importante en las propiedades químicas del suelo (Gallardo y Merino 1993; Vinton y Burke 1995; Cornelissen *et al*. 1999), es esperable que la restauración pasiva y la activa de la vegetación influyan de forma diferente en las propiedades edáficas.

Figura 3. Campo agrícola mediterráneo abandonado bajo sucesión secundaria o restauración pasiva (izquierda) y campo agrícola restaurado de forma activa con *Quercus ilex* (derecha).

Los efectos de la restauración activa de la vegetación en la recuperación de las propiedades químicas del suelo pueden ser tanto positivos como negativos si los comparamos con los de la sucesión secundaria. Por un lado, la introducción de especies leñosas supone un aporte continuo de materia orgánica (Gallardo 2003; Moreno *et al*. 2007) y genera un microclima más favorable para la actividad microbiana y la descomposición de la materia orgánica (Muscolo *et al.* 2007;

Sariyildiz 2008). No obstante, en el caso de especies esclerófilas o resinosas (las más utilizadas en proyectos de restauración), esta materia orgánica es de baja calidad debido a su alto contenido en metabolitos secundarios tales como lignina y tanina, lo que ralentiza su descomposición (Aerts y Chapin 2000; Satti *et al*. 2003). Además, la vegetación leñosa dificulta el crecimiento de las hierbas bajo su copa, que aportan una materia orgánica de elevada calidad (Carrera *et al*. 2009). Cabe, por tanto, preguntarse ¿cuál es el balance en las propiedades químicas del suelo?

Objetivos de la Tesis Doctoral

Esta Tesis Doctoral se enmarca dentro del proyecto Restauración ecológica de áreas degradadas en ambientes mediterráneos continentales. Optimización del uso del agua (M.E.C. Ref. CGL2004-00355/BOS). El objetivo general de esta Tesis es mejorar la restauración de campos agrícolas abandonados en ambientes mediterráneos continentales, donde el déficit hídrico es el principal factor limitante para el establecimiento de las especies leñosas. La hipótesis de partida es que la utilización de plantas de calidad y de técnicas que faciliten el establecimiento de los plantones incrementará el éxito de la restauración de estos campos. En cuanto a la calidad de planta, es de esperar que a escala intraespecífica los fenotipos *productivos* presenten mayor supervivencia y crecimiento que los fenotipos *xeromorfos* y que esta diferencia dependa del nivel de estrés durante el período de establecimiento. En ambientes de estrés hídrico leve o moderado, los fenotipos *productivos* presentarán mayor supervivencia y crecimiento que los *xeromorfos*, mientras que en condiciones severas de estrés hídrico fenotipos *xeromorfos*, con menor consumo de agua, serán los que se establezcan mejor (Fig. 4).

En esta Tesis el proceso de restauración se aborda desde un enfoque multiescala ya que 1) existen varias fases en un proyecto de restauración de la vegetación, tal como se ha mencionado previamente y 2) los niveles de organización implicados son diferentes, incluyendo la respuesta ecofisiológica de las plantas introducidas, su supervivencia y crecimiento, las interacciones de facilitación y competencia entre plantas, la estructura de la comunidad "restaurada" y la función ecosistémica.

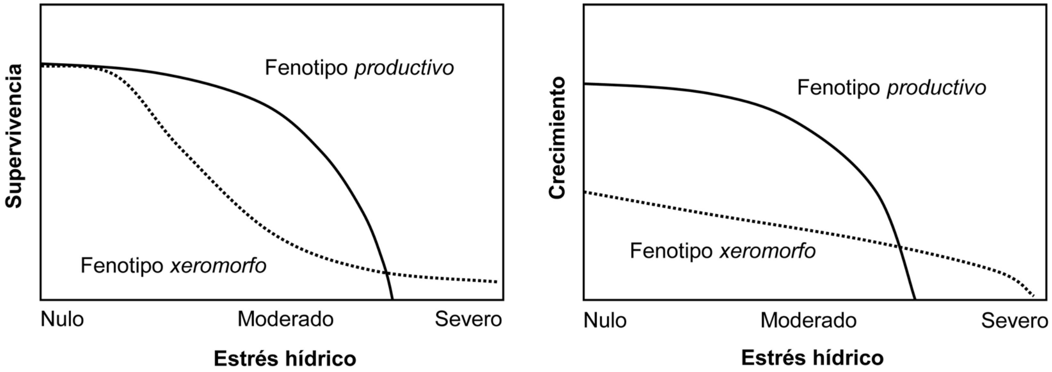

Figura 4. Hipótesis de trabajo relativa a la influencia que tendrán las características funcionales de las plantas en su supervivencia y crecimiento en función del nivel de estrés hídrico del lugar de plantación. Fenotipo *productivo* hace referencia a plantas de parte aérea grande, y con una proporción entre la masa aérea y radical y concentración de nutrientes elevada dentro del rango de variación potencial de la especie a una edad concreta, mientras que fenotipo *xeromorfo* hace referencia a plantas con los atributos funcionales contrarios.

Los objetivos específicos que permiten abordar el objetivo general propuesto son los siguientes:

1. Estudiar los mecanismos ecofisiológicos que explican por qué las plantas grandes y con gran contenido de nitrógeno se establecen mejor en ambientes mediterráneos que plantas con los atributos contrarios.

2. Analizar la interacción entre las características funcionales de los plantones (tamaño y estado nutricional) y el nivel de estrés del ambiente de plantación.

3. Analizar la utilización de *Retama sphaerocarpa* como arbusto nodriza en proyectos de restauración y evaluar el papel que la facilitación indirecta juega en el establecimiento de especies leñosas en retamares mediterráneos.

4. Estudiar la respuesta de las encinas introducidas a la interrupción del manejo aplicado durante los primeros años tras su establecimiento.

5. Conocer los efectos que la restauración pasiva y la revegetación de tierras agrícolas tiene en las propiedades químicas del suelo.

Las especies elegidas para realizar esta investigación han sido la encina, *Quercus ilex*, y el pino carrasco, *Pinus halepensis*. Se han seleccionado estas especies por varias razones. Por un lado, son especies dominantes en muchos ecosistemas forestales mediterráneos y han sido las especies más utilizadas para la restauración de campos agrícolas abandonados en zonas mediterráneas (Zavala *et al*. 2000; MAPA 2006). Por otro lado, estas especies representan tipos funcionales contrastados, con diferentes estrategias para afrontar el estrés hídrico. La encina es una especie de crecimiento lento, característica de etapas tardías de la sucesión, tolerante a la sombra y que resiste la sequía por medio de una estrategia de "derroche" de agua (*sensu* Levitt 1980), por lo que presenta grandes variaciones en su potencial hídrico. El pino carrasco, sin embargo, es una especie pionera de crecimiento rápido, intolerante a la sombra y que resiste la sequía por medio de una estrategia de "ahorro" del agua, por lo que muestra poca variación en su potencial hídrico (Zavala *et al*. 2000; Baquedano y Castillo 2006). Es esperable que dos especies con estrategias fisiológicas contrastadas respondan de forma diferente a la restauración activa.

Estructura de la memoria de la Tesis Doctoral

Esta memoria comprende ocho capítulos. Tras este capítulo introductorio (**capítulo 1**), se presentan cinco capítulos con los resultados de cuatro experimentos y un estudio observacional (**capítulos 2-6**). Estos capítulos se corresponden con artículos científicos publicados o en revisión en revistas científicas internacionales, por lo que su estructura se ha mantenido fiel a la versión para su publicación (se presentan en inglés con sus correspondientes apartados de Resumen, Introducción, Material y métodos, Resultados, Discusión y Bibliografía). Cada uno de estos capítulos se acompaña también de un resumen en español. A continuación, se desarrolla una discusión que integra los resultados de toda la investigación (**capítulo 7**) y, para terminar, se presentan las conclusiones más relevantes de esta Tesis Doctoral (**capítulo 8**).

En el **capítulo 2**: **¿Por qué las plantas grandes, ricas en nitrógeno resisten mejor condiciones estresantes durante el establecimiento? Un análisis fisiológico en dos especies forestales mediterráneas de características funcionales contrastadas** se estudia la supervivencia, crecimiento y comportamiento fisiológico de plantones de *Q. ilex* y *P. halepensis* de morfología y estado nutricional contrastados en dos escenarios de competencia herbácea (**objetivos específicos 1 y 2**). La hipótesis de trabajo es que las plantas grandes y elevada concentración de nutrientes presentan mayor supervivencia y crecimiento que las plantas con características opuestas debido a un mayor crecimiento radical, potencial hídrico, intercambio gaseoso y uso del N.

En el **capítulo 3**: **Crecimiento radical y su interferencia con la elongación aérea en plantones de pino carrasco (*Pinus halepensis* Mill.): efectos del tamaño de planta y la concentración de nitrógeno** se analiza el efecto del tamaño de planta y la concentración de N en la dinámica radical en campo y su interferencia con la elongación aérea en plantones de *P. halepensis* Mill. (**objetivo específico 1**). Las hipótesis de partida son 1) que tanto el tamaño como la concentración de N tienen un efecto positivo en la masa, longitud, profundidad y velocidad de crecimiento del sistema radical, y 2) el compromiso en la asignación de recursos entre parte aérea y parte radical será menor en los individuos con mayor tamaño inicial y mayor concentración de N.

En el **capítulo 4**: **La facilitación de encinas en matorrales mediterráneos está explicada por interacciones directas e indirectas mediadas por hierbas** se aborda la interacción entre un arbusto facilitador (*Retama sphaerocarpa*), la vegetación herbácea y plantones de *Q. ilex* de características funcionales distintas (tamaño y estado nutricional) (**objetivos específicos 1, 2 y 3**). Las hipótesis planteadas fueron: 1) la facilitación indirecta junto con la facilitación directa juega un papel importante en el establecimiento de especies leñosas mediterráneas bajo retama, y 2) en un escenario de facilitación las diferencias de supervivencia y crecimiento entre plantones de distinta calidad serán menores que en un escenario de no facilitación.

En el **capítulo 5**: **Los rasgos de vida en la encina responden de forma diferente a ambientes expereimentales previos** estudia la supervivencia, crecimiento y producción de semillas en una antigua plantación experimental de *Q. ilex* de 13 años, en la que durante los tres primeros años se aplicaron tratamientos facilitadores (una combinación de sombra artificial y riego en verano) que fueron interrumpidos posteriormente. Se compararon los efectos directos del manejo previo con los efectos indirectos de la competencia intraespecífica y los compromisos del reparto de biomasa entre distintas funciones básicas de las plantas (**objetivo específico 4**). Las hipótesis de trabajo fueron: 1) los tratamientos facilitadores aplicados durante el establecimiento de *Q. ilex* mejoran su éxito futuro, aunque la respuesta al ambiente pasado puede ser diferente según el rasgo de vida analizado; 2) el crecimiento radical y el crecimiento aéreo están sujetos a compromisos de asignación de recursos, debido al carácter limitado de los recursos; y 3) la asignación de recursos al crecimiento o a la reproducción supone un compromiso para las plantas por lo que los individuos reproductores crecerán menos que los individuos no reproductores.

En el **capítulo 6**: **Restauración de las propiedades químicas del suelo en un campo agrícola mediterráneo abandonado tras la sucesión secundaria y la reforestación** se comparan las propiedades químicas del suelo en un cultivo activo, una parcela bajo sucesión secundaria durante 13 años, una parcela reforestada de forma activa con *Q. ilex* hace 13 años (la misma que en el capítulo 5) y un encinar maduro de referencia. Se analiza además el efecto que las condiciones ambientales previas y actuales tienen sobre las propiedades químicas del suelo (**objetivo específico 5**). Las hipótesis planteadas fueron tres: 1) las propiedades químicas edáficas de las parcelas restauradas (tanto de forma activa como pasiva) se encuentran en una posición intermedia entre las mismas propiedades del cultivo activo y el encinar de referencia; 2) la restauración activa acelera la recuperación de las propiedades químicas del suelo en comparación con la sucesión secundaria o restauración pasiva; y 3) las propiedades químicas del suelo están determinadas tanto por las condiciones ambientales previas como por las condiciones actuales.

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Los cambios pueden ocurrir naturalmente o de forma brusca,
obligándonos a salir de los caminos conocidos y cómodos.
¿Atreverse o no atreverse a salirse de ese camino?
Algunos prefieren perder ese sentimiento de contacto con el suelo...
y descubrir la liberación de un cielo abierto.
Gracias por atreverte.

Virginia Hernández Santana

Capítulo 2

Capítulo 2

¿Por qué las plantas grandes, ricas en nitrógeno resisten mejor condiciones estresantes durante el establecimiento? Un análisis fisiológico en dos especies forestales mediterráneas de características funcionales contrastadas

Este capítulo reproduce íntegramente el texto del siguiente manuscrito:

Cuesta, B., Villar-Salvador, P., Puértolas, J. Jacobs, D. and Rey-Benayas, J.M. Why do large, nitrogen rich seedlings better resist stressful transplanting conditions? A physiological analysis in two functionally contrasting Mediterranean forest species. En revisión en Forest Ecology and Mangement.

Resumen

Se analiza la base fisiológica que explica por qué los plantones grandes y los plantones con gran concentración de nitrógeno (N) tienen frecuentemente mayor supervivencia y crecimiento que los plantones pequeños en plantaciones forestales mediterráneas. Se introdujeron plantones grandes de pino carrasco (*Pinus halepensis* Mill.) y encina (*Quercus ilex* L.) con gran concentración de N (*L*+) y plantones pequeños tanto con alta (*S*+) como con baja (*S*-) concentración de N en dos sitios con diferente intensidad de competencia herbácea y, por tanto, condiciones de estrés contrastadas. Se midió la supervivencia, el crecimiento, el intercambio gaseoso, la remobilización (N_R) y absorción (N_U) de N y el potencial hídrico de los plantones durante la primera estación de crecimiento. Las hierbas redujeron la supervivencia y el crecimiento, pero la respuesta de los plantones varió entre fenotipos y entre especies. Al final de la primera estación de crecimiento, los plantones de pinos *L*+ sobrevivieron más que los dos tipos de plantones pequeños en presencia de hierbas, pero no se observaron diferencias en ausencia de hierbas. Las diferencias de mortalidad entre fenotipos ocurrieron en primavera pero no en verano. Los pinos *L*+ crecieron más que los pinos pequeños independientemente de la competencia herbácea. Ningún plantón de encina sobrevivió en presencia de hierbas y no se observaron diferencias de mortalidad entre fenotipos en ausencia de hierbas, aunque los plantones de encina *L*+ crecieron más que los plantones pequeños. Las diferencias de mortalidad y crecimiento estuvieron ligadas a diferencias fisiológicas. Los fenotipos de pino presentaron importantes diferencias fisiológicas, mientras que las diferencias entre los fenotipos de encina fueron pequeñas. Los pinos *L*+ tuvieron mayor crecimiento radical, intercambio gaseoso, N_R y N_U que los plantones pequeños independientemente de su concentración de N. Concluimos que las diferencias funcionales entre plantones tuvieron consecuencias diferentes para el establecimiento en el campo de ambas especies. El mejor establecimiento de los pinos *L*+ en comparación con los pequeños estuvo ligado a un mayor intercambio gaseoso, crecimiento radical y uso del N. El tamaño de los plantones tuvo un papel más importante en el establecimiento de los plantones que la concentración de N.

Palabras clave: competencia, conductancia estomática, crecimiento radical, fotosíntesis, *Pinus halepensis*, potencial hídrico, *Quercus ilex*, removilización de N, supervivencia.

Why do large, nitrogen rich seedlings better resist stressful transplanting conditions? A physiological analysis in two functionally contrasting Mediterranean forest species

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Abstract

We analysed the physiological basis that explain why large and high nitrogen (N) concentration seedlings frequently have improved survival and growth relative to small seedlings in Mediterranean woodland plantations. Large seedlings of Aleppo pine (*Pinus halepensis* Mill.) and holm oak (*Quercus ilex* L.) with high N concentration (*L*+), and small seedlings with either high (*S*+) or low (*S*-) N concentration, were planted on two sites of different herb competition intensity that created contrasting stress conditions. Seedling survival, growth, gas exchange, N remobilisation (N_R) and uptake (N_U), and water potential were assessed through the first growing season. Herbs reduced survival and growth, but seedling response varied among phenotypes and between species. At the end of the first growing season, *L*+ pine seedlings survived more than both small seedling types in presence of herbs but no differences were observed in absence of herbs. Mortality differences among phenotypes occurred in spring but not in summer. *L*+ pines grew more than small pines independently of herb competition. No oak seedling type survived in presence of herbs and no mortality differences among phenotypes where observed in absence of herbs, although *L*+ oak seedlings grew more than small seedlings. Mortality and growth differences were linked to physiological differences. Pine phenotypes had marked physiological differences while differences were small in oak phenotypes. *L*+ pines had greater root growth, gas exchange, N_R and N_U than small seedlings, irrespective of their N concentration. We conclude that the functional differences among seedlings had different consequences for transplanting performance in both species. Improved transplanting performance in large pine seedlings relative to small plants was linked to greater gas exchange, root growth and N cycling. Seedling size had a greater role in the performance of transplanted seedlings than N concentration.

Key words: competition, nitrogen remobilisation, *Pinus halepensis*, photosynthesis, *Quercus ilex*, root growth, stomatal conductance, survival, water potential.

Introduction

Water stress caused by herb competition and summer drought are major factors that limit establishment of planted seedlings in abandoned Mediterranean croplands (Rey Benayas *et al*. 2005). Seedling transplanting performance also depends on seedling morphological and physiological attributes, which can be determined to a great extent by cultivation practices in the nursery (van den Driessche 1991a; Villar-Salvador *et al*. 2004). Much evidence indicates that, for a given species, large seedlings frequently have improved survival and growth relative to small seedlings in Mediterranean environments (Puértolas *et al*. 2003; Villar-Salvador *et al*. 2004; Tsakaldimi *et al*. 2005; Navarro *et al*. 2006; Villar-Salvador *et al*. 2008; Luis *et al*. 2009; Oliet *et al*. 2009). Similar trends have also been reported in boreal and humid temperate environments, where large seedlings are more competitive against herbs than small seedlings (Lamhamedi *et al*. 1998; Noland *et al*. 2001; South *et al*. 2005). However, large seedlings in some instances may have lower performance than small seedlings in very dry reforestation sites (Rose *et al*. 1993; Trubat *et al*. 2008). Several studies have also reported a positive correlation between transplanting performance of forest species and plant nitrogen (N) concentration (van den Driessche 1988; Puértolas *et al*. 2003; Villar-Salvador *et al*. 2004; Oliet *et al*. 2009). This indicates that seedling nutrient composition can play an important role in transplanting performance (Malik and Timmer 1996; Salifu and Timmer 2003), probably due to remobilisation of internal N reserves (Millard *et al*. 2001; Silla and Escudero 2003).

The above-mentioned relationships provide some insights into potential physiological mechanisms involved in the greater transplanting performance of large or high N concentration seedlings. As far as we know, however, no published studies have addressed the physiological mechanisms underlying such relationships. Based on the transplant shock model of Burdett *et al*. (1990), Villar-Salvador (2003) proposed a conceptual model to explain the influence of initial plant size and N concentration on seedling survival in Mediterranean environments. This model suggests that large seedlings and seedlings with high N concentration have higher net carbon gain because they have greater photosynthetic surface and photosynthesis rate (Field and Mooney 1986; Jose *et al*. 2003), respectively, than small seedlings or plants with low N concentration. Increase in carbon gain stimulates root growth (van den Driessche 1991b) during the wet season, allowing seedlings to extract water and nutrients from a greater soil volume during the dry season. This allows plants to maintain high water potential and positive carbon balance (Burdett *et al*. 1983), which is critical to ensure plant survival during the dry season (Padilla and Pugnaire 2007). Thus, optimal seedling attributes for Mediterranean dry environments should promote high photosynthesis and nutrient remobilisation during the wet season to ensure suitable water and carbon balance during the dry season.

The general objective of this study was to study the physiological mechanisms underlying the higher transplanting performance of large seedlings and seedlings with high N concentration in Mediterranean reforestation sites. Specifically, we compared the physiological performance of Aleppo pine (*Pinus halepensis* Mill.) and holm oak (*Quercus ilex* L.) seedlings of contrasting size and N concentration transplanted into two environments that differed in stress conditions. Distinct stressful transplanting conditions were achieved by manipulating herb density, which induced marked differences in soil water availability. Seedling survival, growth and physiological performance were assessed in late spring at the end of the establishment period when drought stress was low, and in mid-summer when drought stress was pronounced. We selected *P. halepensis* and *Q. ilex*, which are widely used in afforestation in the Mediterranean basin, to test if the performance of contrasting seedling phenotypes differed between species that have different functional strategies to deal with environmental stresses. The oak is a shade-tolerant and slow growing species characteristic of late forest successional stages that closes stomata at low tissue water potential (Ψ) thereby tolerating low tissue relative water content (RWC). By contrast, the pine is a fast growing shade-intolerant pioneer species that closes stomata at high tissue Ψ and does not tolerate low tissue RWC (Zavala *et al*. 2000; Baquedano and Castillo 2006).

Material and methods

Seedling cultivation

Seeds originating from inland Spain provenances (Alcarria-Serranía de Cuenca and Alcarria for *Q. ilex* and *P. halepensis*, respectively) were sown in Plasnor 190/300-45 trays (45 cavities of 300 ml per tray; Plasnor, Legazpi, Spain) with unfertilized *Sphagnum* peat (Vapopeat XL, Vapo, Jyväskylä, Finland). We cultivated 1080 seedlings of three phenotypes in both species that differed in their morphology and N concentration (Table 1): (1) large seedlings with high N concentration (*L*+), (2) small seedlings with high N concentration (*S*+), and (3) small seedlings with low N concentration (*S*-). These differences were achieved by varying the length of the growing period and fertilization rate. *L*+ seedlings were sown on December 15, 2004 and fertilized twice weekly with a fertilizer solution of 100 ppm N from late May to mid December 2005. Small seedlings were sown on March 21, 2005 and fertilized weekly with 100 ppm N from late May until mid July 2005. Subsequently, fertilization of small seedlings was withheld for three weeks until early August and resumed until the end of October 2005, with seedlings being fertilized fortnightly with 50 ppm N. Half of the seedlings sown in March then received no fertilization until the end of the cultivation (*S*- seedlings) while the remainder were heavily fertilized once per week with 200 ppm N (*S*+ seedlings). Fertilization was done with a Peters Professional® 20-7-19: N-P_2O_5-K_2O fertilizer (Scotts, USA). Furthermore, *L*+ and *S*+ seedlings were weekly sprayed with a foliar fertilizer (N-P-K: 8-8-6, Wuxal AA, Schering España, S.A., Madrid, Spain) at a 250 ml l^{-1} concentration for three weeks in December 2005. Plants were arranged in space in four random blocks. To avoid frost damage, plants were initially grown in a glass greenhouse in which temperature ranged from 4 to 25 ºC and radiation was approximately 50% of that outside. In mid May 2005, seedlings were moved outside. Plants were kept well watered according to their requirements by irrigating them every 1-3 days. Cultivation was completed in the third week of December 2005.

Morphology and N concentration measurements following cultivation

At the end of the cultivation period, 12 and 16 plants per phenotype in *P. halepensis* and *Q. ilex*, respectively, were randomly sampled and their shoots were cut at the cotyledon insertion point and root plugs were cleaned from the peat. Shoot height was measured as the length of the stem while shoot diameter was measured as the stem thickness at the cotyledon insertion point. Then, all plant parts were washed with tap water, rinsed in distilled water and dried in a ventilated oven at 60ºC for 48 h to measure their mass.

To assess tissue N concentration, plants were randomly distributed into four groups and their shoots and roots were pooled separately and finely ground. N concentration was determined by Kjeldahl analysis with K-SeSO$_4$-Se$_2$Cu$_4$ as catalyst in a Tecator DS-40 digestion system (FOSS Tecator, Sweden) and a SAN ++ autoanalyser (Skalar, Netherlands).

Field study site and plantation experimental design

The field study site was located in Guadalajara, central Spain (40º39´ N, 3º10´ W) at 650 m a.s.l. on a flat river terrace with a poorly developed *Entisol* soil from alluvium material. The climate is Mediterranean continental with mean annual precipitation and temperature of 400 mm and 13.4 ºC, respectively. Winter mean air temperature is < 6.5 ºC. Spring (from March to late May) is usually mild and wet. Mean spring rainfall and mean temperature of the last 10 years were 118 mm and 12.4 ºC, respectively. A pronounced dry and hot season occurs from June to mid September, where total rainfall is 47 mm and mean maximum temperatures is 31ºC. Spring rainfall and mean air temperature in the study year (2006) were 119 mm and 12.8 ºC, respectively, while summer rainfall and mean maximum air temperature were 51 mm and 31.5 ºC, respectively.

Seedlings were transplanted to the field on late December 2005. The experimental design consisted of a split-plot with five blocks. Each block was split into two plots or main plots, which were randomly assigned to each herb competition treatment (presence and absence of herbs). In the herb treatment plot, the natural herb community was allowed to develop while in the other plot herbs were removed with herbicide (Oxifluorfen 24%, Iteike TradeCorp, Spain) at planting and at mid-summer along with manual hoeing when necessary. In each main plot, we randomly differentiated six subplots in which six treatments resulting from the combination of two species x three seedling phenotypes were randomly assigned. In each subplot, we planted a row of 12 seedlings per treatment. Distance between seedlings within a row was 1 m and distance between subplots was 2 m. Soil was subsoiled to 50 cm in depth before planting. Dominant species in the herb plots were *Avena sterilis*, *Bromus* spp., *Capsella bursa-pastoris*, *Chenopodium album*, *Hordeum murinum*, *Papaver rhoeas*, *Portulaca oleracea*, *Rapistrum rugosum*, *Salsola kali*, and *Taeniathezum caput-medusae*.

Effect of herbs on seedling microclimate

Incident photosynthetic active radiation at seedling mid-height was measured at midday in May on a clear day using a quantum photo/radiometer (HD 9021, Delta OHM, Casselle Di Selvazzano, Italy) in three seedlings per treatment and block. Air vapour pressure deficit (VPD) was quantified in late spring and in mid-summer from the air relative humidity and temperature measures taken next to five seedlings per treatment using a portable gas exchange system (LCpro+ System, ADC BioScientific, Hoddesdon, UK). Soil water content in the 0-60 cm soil profile was measured monthly from April to September by Time Domain Reflectometry (TDR, Tektronix, Beauverton, CO, USA) according to methodology in Cassel *et al*. (1994). We made one measurement per herb treatment and block. In late May, when flowering of the herbaceous community was completed, we measured herb height and aboveground mass contained in three 50 x 50 cm quadrants per block after drying at 50 ºC for two days.

Transplant mortality and shoot growth

Seedling mortality and shoot growth was recorded at the end of spring (last week of May) and after summer drought (last week of October) of 2006. We measured the spring (from February to June), summer (from June to October) and total first growing season (from February to October) seedling mortality and seedling shoot growth. Shoot growth was measured as the stem volume increase and calculated as the difference between the stem volume at the end and at the beginning of each experimental period (spring and summer). Stem volume was calculated from the height and diameter of the stem assuming that the stem of the seedling is a cone. Diameter was measured at the cotyledon insertion point.

Gas exchange and water potential measurements in the field

We compared the net photosynthesis rate (A), stomatal conductance to water vapour (g_s), and Ψ of seedling phenotypes in spring (late May), before summer drought onset, and in mid-summer (late July), the hottest and driest moment of the year, of 2006. In spring after the first shoot flush of growth and when leaves were mature, A and g_s were measured in the morning and at midday (from 9:00 to 11:00 h and from 12:00 to 14:00 h solar time, respectively) on current year shoots. Ψ was measured at pre-dawn and at midday on current year leaves (in oak) or twigs (in pine). In summer, we could not measure all treatments because no oak seedling survived in the herb plot. In plots with absence of herbs, pre-dawn Ψ was measured only in *P. halepensis*, while midday Ψ was measured in both species. In the herb plots, summer A and g_s in the morning and at midday, and midday Ψ in *P. halepensis* were only measured in *L*+ and *S*+ phenotypes due to low number of surviving *S*- seedlings. Gas exchange was measured with a portable gas exchange system (LCpro+ System, ADC BioScientific, Hoddesdon, UK). Shoots were enclosed in the conifer chamber-type until measurement stabilisation, which usually took 3-4 minutes. Ψ was measured with a Scholander-type pressure chamber. In all cases, we made measurements in one randomly selected plant per treatment and block (six plants per treatment). In spring, air temperature and VPD at measuring time were 29.3 ± 0.4 ºC and 3.1 ± 0.11 KPa (mean ± 1 SE) in the morning and 37.1 ± 0.5 ºC and 5.5 ± 0.12 KPa at midday. In summer, air temperature and VPD were 29.3 ± 0.5 ºC and 3.0 ± 0.16 KPa in the morning and 41.0 ± 0.5 ºC and 6.8 ± 0.22 KPa at midday.

Root growth and N cycling

In late May 2006, the plants used for gas exchange measurements were extracted by carefully digging a 50 cm deep hole using an excavator with a 0.04 m^3 bucket. Plants were separated into old and new shoots and roots and senescent leaves, washed with tap water, rinsed in distilled water, and dried in a ventilated oven at 60 ºC for 48 h for organ mass determinations. Most seedlings had no senescent leaves, or if present they remained attached to the stem in both species. Roots protruding out of the root plug were designated as new roots while those in the root plug were classified as old roots.

Plant fractions were finely ground and N concentration was determined by Kjeldahl analysis with K-$SeSO_4$-Se_2Cu_4 as catalyst in a Tecator DS-40 digestion system (FOSS Tecator, Sweden) and a SAN ++ auto-analyser (Skalar, Netherlands). The N content of each fraction was calculated as the product of the N concentration and the mass of the fraction. The N remobilised (N_R) from old tissues and net N uptake (N_U) from soil was estimated following similar methodology in Silla and Escudero (2003). Briefly, N_R was calculated as

$$N_R = N_p - N_o - N_s \quad \text{(mg)} \qquad (1)$$

where N_p, N_o, and N_s are the plant N content at the end of the cultivation, N content in old roots and shoots, and N content in senescent leaves, respectively. N_U was quantified as

$$N_U = (N_o + N_n) - N_p - N_s \quad \text{(mg)} \quad (2)$$

where N_n is the N content in new shoots and roots.

Standardization of N_R by plant N content at planting was used as a measure of N remobilisation efficiency.

Data analysis

Although the experimental design included species as a factor, we performed statistical analyses separately for each species due to strong heterogeneity of variance, which could not be corrected by data transformation. The effect of herb competition and seedling type in irradiance were analysed by two-way ANOVA, and the effect of herb competition in air temperature and VPD were analysed by one-way ANOVA. Soil water content was analyzed by repeated measures ANOVA. Differences in seedling attributes at the end of nursery cultivation were analysed by one-way ANOVA. Spring (February to late May), summer (June to late October) and cumulative (February to late October) mortality were analysed using a generalized linear model with a binomial distribution and a logit link function. Block, herb treatment and seedling type were the factors. Seedling shoot growth were analysed by a split-plot analysis with measures over time (Gómez and Gómez 1984). New root production, N remobilization and N uptake in both species were analysed by split-plot analysis (Gómez and Gómez 1984). *A*, g_s, and Ψ of both species in spring (May) and of *P. halepensis* in summer were analysed by a split-plot analysis with measures over time. In summer, as no *S*- pine seedling was measured in the herb competition plots, seedling type was nested to herb competition for *P. halepensis*. Oak *A* and g_s in summer were analysed by repeated measures ANOVA, whereas midday Ψ was analysed by two-way ANOVA. Data were checked for normality and homogeneity of variances, and were transformed when necessary to correct deviations from these assumptions. All statistical analyses were performed with the Statistica 6.0. Package (StatSoft, Inc., Tulsa, OK, USA).

Results

Functional characteristics of phenotypes following cultivation

Shoot height and diameter in *L*+ *P. halepensis* seedlings were 2.4 and 1.4 times higher, respectively, than in *S*+ and *S*- seedlings, which did not differ between them. Similarly, shoot and root mass in *L*+ seedlings were four and two times higher, respectively, than in *S*+ and

Table 1. Morphological attributes, N concentration and N content of the three seedling phenotypes of *P. halepensis* and *Q. ilex* at planting. Data are means ± 1 SE; n=16 for morphological attributes and n=4 for N data. *L*+: large seedlings with high N concentration, *S*+: small seedlings with high N concentration, and *S*-: small seedlings with low N concentration Seedling phenotypes with different letter indicate significant differences at *P* < 0.05 after a *post-hoc* Tukey´s test.

	P. halepensis			*Q. ilex*		
	L+	*S+*	*S-*	*L+*	*S+*	*S-*
Plant height (cm)	18 ± 1.6^{a}	7.5 ± 0.3^{b}	7.5 ± 0.4^{b}	15 ± 1.1^{a}	11 ± 0.7^{b}	11.5 ± 0.9^{b}
Plant diameter (mm)	3.1 ± 0.1^{a}	2.2 ± 0.1^{b}	2.2 ± 0.1^{b}	4.0 ± 0.2^{b}	3.3 ± 0.2^{b}	3.5 ± 0.3^{b}
Shoot mass (g)	4.0 ± 0.3^{a}	0.9 ± 0.1^{b}	1.1 ± 0.1^{b}	1.9 ± 0.2^{a}	1.1 ± 0.1^{b}	1.1 ± 0.2^{b}
Root mass (g)	2.6 ± 0.3^{a}	1.0 ± 0.1^{b}	1.3 ± 0.1^{b}	2.1 ± 0.2^{a}	1.3 ± 0.2^{b}	1.8 ± 0.3^{ab}
Plant N concentration (mg g^{-1})	22 ± 1.2^{a}	19 ± 0.8^{a}	9 ± 0.2^{b}	18 ± 0.3^{a}	13 ± 0.3^{b}	9 ± 0.5^{c}
Plant N content (mg)	154 ± 10^{a}	37 ± 3^{b}	22 ± 2^{b}	71 ± 9^{a}	32 ± 3^{b}	27 ± 2^{b}

S- seedlings, which did not differ between them. N concentration of *L*+ seedlings was similar to that of *S*+ seedlings but it was 2.3 times higher than that of S- seedlings. *S*+ seedlings had twice the N concentration of *S*- seedlings (Table 1).

Seedling microenvironment in the field

Mean height and aboveground biomass of the herbaceous vegetation was 65 ± 5 cm and 0.22 ± 0.02 kg m^{-2}, respectively. Herbs reduced incident photosynthetic active radiation by 25%, but reduction in irradiation did not differ among seedling types (data not shown). Air temperature was lower in presence than in absence of herbs (36.7 ± 0.4 *vs*. 40.1 ± 0.9, respectively, in spring and 37.7 ± 0.5 ºC *vs*. 41.3 ± 0.5 ºC, respectively, in summer), but these differences were not statistically significant. Herbs did not modify VPD neither in spring (4.3 ± 0.18 and 4.4 ± 0.19 KPa in presence and absence of herbs, respectively) nor in summer (4.5 ± 0.55 and 5.0 ± 0.29 KPa in presence and absence of herbs, respectively). Soil water content in mid-spring was 13% and diminished through summer until 9% (*P* < 0.001). Herbs reduced soil water content by *ca*. 50% in spring and early summer but differences between herb and control plots dissipated in mid-summer (date × herb competition interaction, *P* < 0.001).

Transplant mortality and shoot and root growth

Seedling mortality was lower in pine than in oak and it was lower in spring than in summer. In both species, herb competition strongly increased mortality in spring (*P* < 0.001 for both, pine and oak) and in summer (*P* < 0.001 for both, pine and oak) (Fig. 1). All oak seedlings died in the herb plots in summer. In pine, *L*+ and *S*- plants had the lowest and highest spring mortality, respectively, having *S*+ plants intermediate values (*P* < 0.001). No differences were observed in mortality among pine seedling phenotypes in summer. Cumulative mortality in *L*+ pine seedlings during the whole first growing

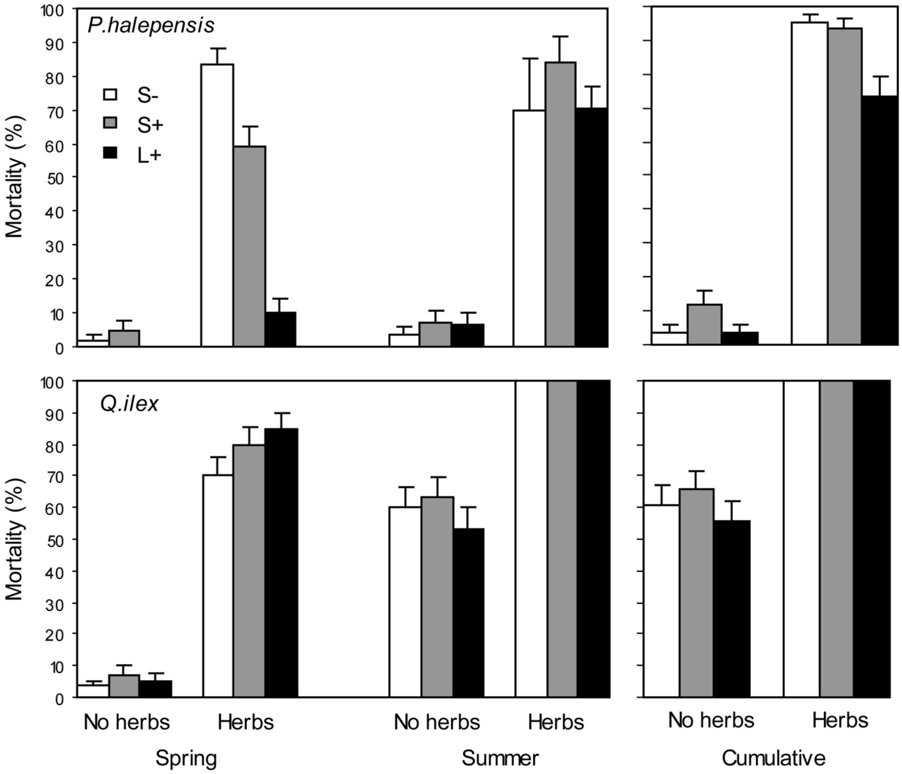

Figure 1. Mortality of *P. halepensis* (above) and *Q. ilex* (below) seedlings transplanted with herb and without herb competition during spring, summer and the whole first growing season (cumulative). In *Q. ilex*, no seedling survived in the herb plots during summer. Data are means ± 1 SE. *L*+: large seedlings with high N concentration, *S*+: small seedling with high N concentration, and *S*-: small seedlings with low N concentration.

season was lower than either *S*+ or *S*- plants in presence of herbs, whereas no difference among seedling types was found in absence of herbs (seedling type × herb competition interaction, *P* = 0.013). In oak, no differences in mortality were observed among seedling phenotypes in spring, in summer or in cumulative mortality during the first growing season.

Pine seedlings grew more than oak seedlings. Both species grew more in summer than in spring (*P* = 0.04 for pine and *P* < 0.001 for oak). Herbs reduced seedling growth in both species (*P* = 0.042 for pine and *P* = 0.02 for oak) (Fig. 2). *L*+ pines grew more than small pine seedlings, and this difference was greater in absence than in presence of herbs (seedling type × herb competition interaction, *P* = 0.003). *L*+ oak seedlings grew more than small oaks in absence of herbs, but no differences among seedling types was observed in presence of herbs (seedling type × herb competition interaction, *P* = 0.005). Oak seedlings showed negative growth in spring in presence of herbs. In absence of herbs, *L*+ oak seedlings grew more than small oaks in summer but no difference was observed in spring (seedling type × season interaction, *P* < 0.001). In both species, *S*+ plants had greater growth than *S*- seedlings but differences were not statistically significant. In pine, *L*+ seedlings produced more new roots in the field than either *S*+ or *S*- seedlings, and this difference was greater in presence than in absence of herbs (seedling type × herb competition interaction, $P = 0.04$; Fig. 2). Neither herb competition nor seedling phenotype affected new root growth in oak.

Seedling physiological performance in the field

Herbs reduced gas exchange and Ψ in both species ($P < 0.05$, Fig. 3). For instance, predawn Ψ in spring without herbs was higher than -0.8 MPa in both species, but it was -2.3 ± 0.3 and -3.3 ± 0.3 MPa (mean ± 1 SE) for pine and oak, respectively, in the herb plot. In pine, reduction in *A* and g_s by herbs was greater at midday than at morning in summer but not in spring (time of measure × herb competition interaction, $P < 0.03$). Midday *A* was negative in summer and in presence of herbs. In spring and in presence of herbs, *L*+ pine seedlings had higher *A* and g_s than *S*+ and *S*- seedlings, which did not differ between them. However, in absence of herbs seedling types did not differ in gas exchange (seedling type × herb competition interaction, $P < 0.04$). By contrast, *L*+ pines in summer had higher *A* and g_s than small pines in absence of herbs, but in their

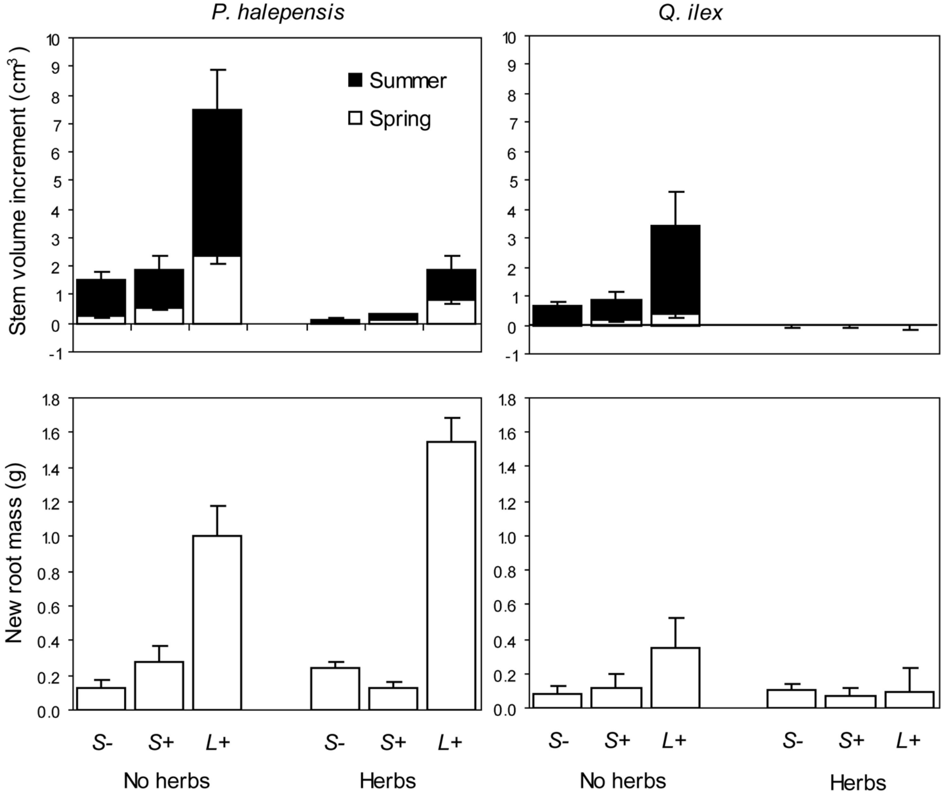

Figure 2. Shoot growth, measured as the stem volume increment, during spring and summer (above) and new root growth in the field (below) during spring of *P. halepensis* (left) and *Q. ilex* (right) seedlings, transplanted with and without herb competition. Data are means ± 1 SE; n=5. *L*+: large seedlings with high N concentration, *S*+: small seedlings with high N concentration, and *S*-: small seedlings with low N concentration.

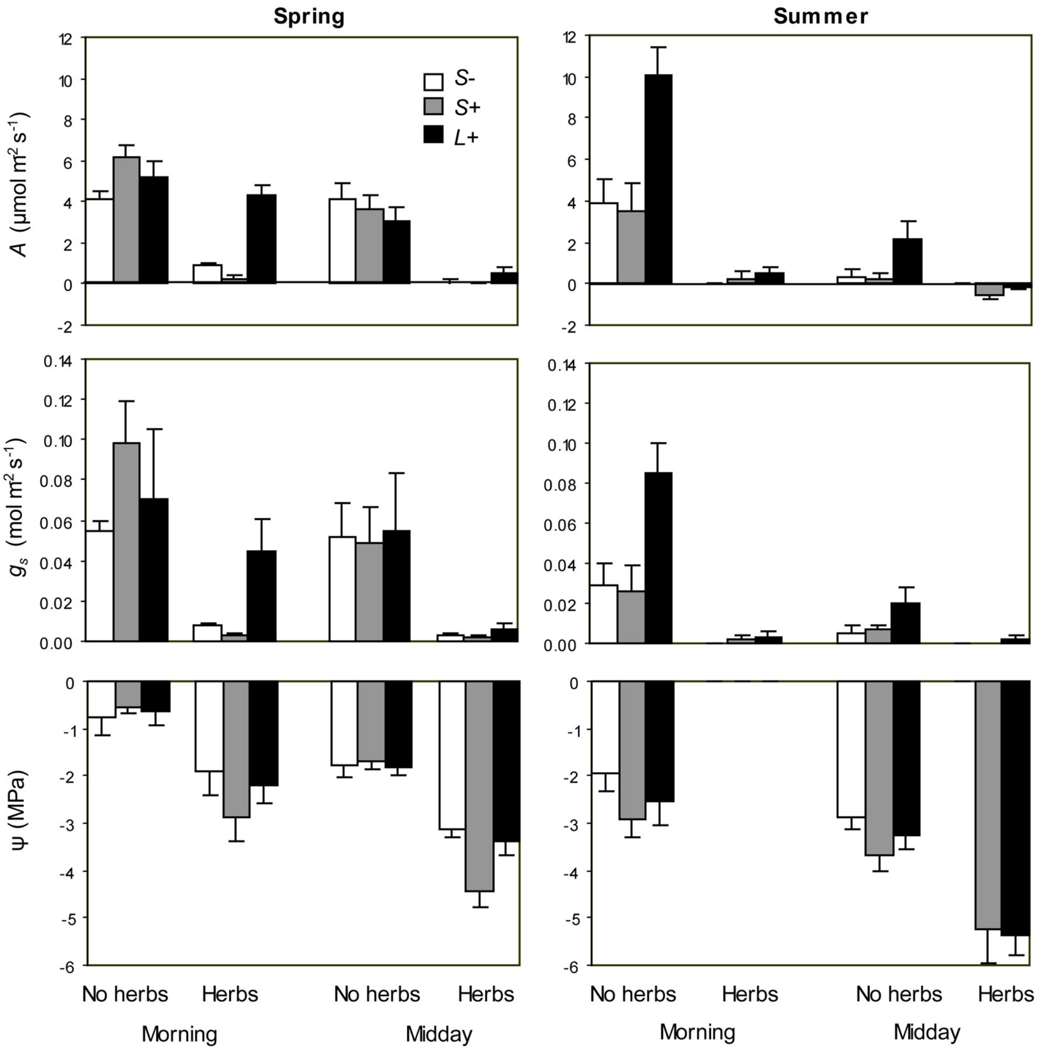

Figure 3. Morning and midday net photosynthesis rate (*A*), stomatal conductance to water vapour (g_S) and water potential (Ψ) in both spring and summer in three *P. halepensis* seedling phenotypes, transplanted with and without herb competition. Data are means ± 1 SE; n=5. *L*+: large seedlings with high N concentration, *S*+: small seedlings with high N concentration, and *S*-: small seedlings with low N concentration.

presence seedling types did not differ in gas exchange (Fig. 3). Neither in spring nor in summer did Ψ differ among pine seedling types. In oak, seedling types did not differ in Ψ or gas exchange, both in spring and in summer.

N_U was greater than N_R in absence of herbs in both species, especially in *S*+ and *L*+ seedlings, but in presence of herbs N_R was greater than N_U (Fig. 4). This variation was due to a reduction in N_U by herbs in both species ($P < 0.001$ for pine and $P = 0.07$ for oak) with no changes in N_R. N uptake by *L*+ pine seedlings was higher than N_U by small seedlings, which did not differ between them. However, this difference was only evident in absence of herbs (seedling type × herb competition interaction, $P = 0.03$). N_R was not affected by herb presence, but it differed among seedling types, being highest in *L*+ plants, lowest in *S*- seedlings and intermediate in *S*+ plants ($P < 0.001$). Relative N_R was similar in *L*+ and *S*+ pines, and higher than in *S*- pines ($P < 0.001$). *L*+ oaks absorbed more N than small seedlings, which did not differ between them ($P = 0.02$). Neither herb competition nor seedling type affected the absolute or relative N_R in oak.

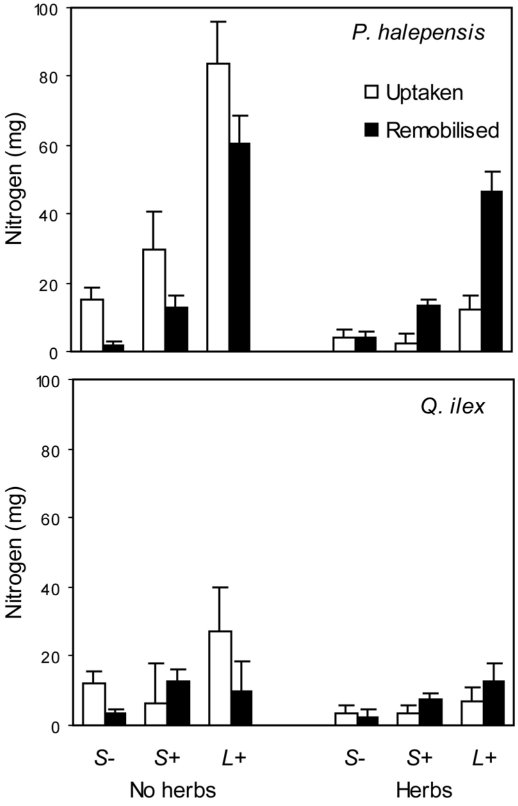

Figure 4. N remobilisation and uptake in three seedling phenotypes of *P. halepensis* (above) and *Q. ilex* (below), transplanted with (right) and without (left) herb competition. Data represent means ± 1 SE; n = 5. *L*+: large seedlings with high N concentration, *S*+: small seedlings with high N concentration, and *S*-: small seedlings with low N concentration.

In pine, N concentration in new shoots did not differ among seedling types in absence of herbs (14 ± 0.6 mg g^{-1} in *S*- to 13 ± 0.9 mg g^{-1} in *S*+). By contrast, in competition with herbs, *S*+ and S- pine seedlings had highest and lowest N concentrations in new shoots (15 ± 1.5 and 9 ± 0.7 mg g^{-1}, respectively), whereas *L*+ plants showed intermediate values (11 ± 1.1 mg g^{-1}) (seedling type × herb competition interaction, $P = 0.006$). In oak, N concentration in new shoots did not differ among phenotypes, neither in presence nor in absence of herbs (data not shown).

Discussion

Herbs induced strong water stress that impaired seedling physiological performance and consequently reduced survival and growth in both species. Similar effects of herbs on forest species have previously been reported (Gordon *et al*. 1989; Löf 2000; Picon-Cochard *et al*. 2006). Seedling phenotypes had marked differences in performance in response to the stressful conditions created by herbs, and these differences depended on species. At the end of the first growing season, large seedlings had greater survival and growth than small seedlings (irrespective of N concentration) in pine, while no oak seedling type could survive. Results for pine conform with the frequent improved performance of large seedlings in Mediterranean woodland plantations (Puértolas *et al*. 2003; Villar-Salvador *et al*. 2004; Tsakaldimi *et al*. 2005; Villar-Salvador *et al*. 2008; Luis *et al*. 2009; Oliet *et al*. 2009). By contrast, under low and mild stress, as in those in plots without herbs, seedling types did not differ in mortality but growth was higher in larger than in smaller plants in both species. Similar findings were reported in *P. halepensis* plantations in eastern Spain (Del Campo García *et al*. 2007). Mortality differences among pine phenotypes in competition with herbs occurred in spring, but not in summer, maybe because environmental conditions were too stressful. This result suggests that better performance of large pine seedlings relative to small seedlings occurs under low and moderate stress but not under severe drought conditions where no phenotype has any advantage.

Physiological differences among seedling pine phenotypes after transplanting

Pinus halepensis phenotypes had marked physiological differences, while differences among oak phenotypes were very small. Thus, we focussed this section on pine. Maintenance of photosynthetic capacity is essential for surviving under resource limiting conditions (Johnson and Smith 2005). Large pine seedlings had higher A and g_s than small seedlings, irrespective of their N concentration, under low (in spring with herb competition) and moderate (in summer without herb competition) stress conditions. This might explain the reduced spring mortality of large pine seedlings relative to small pines in herb plots. In contrast, under severe drought as it occurred in summer in the herb plots, net photosynthesis rate was very low or negative and did not differ among pine phenotypes. Accordingly, summer mortality was very high in the herb plots and did not differ among pine phenotypes.

Production of large and deep roots is important for overcoming water stress (Grossnickle 2005; Padilla and Pugnaire 2007). Large seedlings produced more new roots than small seedlings at the onset of summer drought. Interestingly, herbs stimulated new root growth in large seedlings but not in small seedlings, indicating that large pines had greater capacity to allocated resources to roots in response to stress induced by herbs than small seedlings. Contrary to our expectations, however, greater root development did not provide any advantage in Ψ indicating that greater g_s and A in large pines cannot be ascribed in this study to either differences in Ψ (Kolb and Stone 2000; Luis *et al*. 2009) or foliage N concentration (Samuelson *et al*. 2001; Maier *et al*. 2002), as large pines did not have the highest N concentration in current year shoots. We hypothesize that increased g_s, hence A, in large seedlings compared to small plants may be attributed to greater hydraulic conductivity linked to new root growth (Grossnickle and Russell 1990; Hubbard *et al*. 2001; Sayer *et al*. 2005) or changes in root specific hydraulic conductance (Trubat *et al*. 2006). Teskey *et al*. (1983) showed that partial root removal depressed g_s in *Abies amabilis* independent of leaf water potential.

N remobilisation from storage organs and uptake support plant growth (Millard 1996; Salifu and Timmer 2003; Silla and Escudero 2003). Pine phenotypes differed in N cycling, with large pines remobilising and absorbing more N than small pines. This is consistent with greater shoot and root growth of large plants relative to small ones and highlights the importance of N cycling for growing in high-competition or nutrient poor sites. Increased remobilisation in large seedlings, which contained more N than small seedlings at planting, and the absence of any effect of herbs on remobilisation support the contention that N remobilisation is driven by source rather than sink strength (Millard *et al*. 2001). This response contrasts with that in *Picea mariana* where herb competition stimulated N remobilisation (Malik and Timmer 1996). It is remarkable that seedlings with high tissue N concentration (*i.e.* *L*+ and *S*+ plants) remobilised more N than seedlings with low tissue N concentration not only because they had higher N content but also because they remobilised a higher proportion of their stored N. This might increase N use efficiency of high N concentration seedlings in low-fertility transplanting sites. Similar to our findings, Millet *et al.* (2005) observed a positive relationship between N uptake and sapling size. Differences in N uptake between large and small plants can be attributed to differences in the size and architecture of the new root system (Chapin 1991), growth, which determines sink strength (Nambiar and Fife 1991), or transpiration, which determines nutrient mass flow. Independent of seedling type, herbs reduced N uptake. Reduction in N uptake in presence of herbs might be due either to lower soil N and water availability or to lower seedling growth, which reduces sink strength (Nambiar and Fife 1991; Picon-Cochard *et al*. 2006).

Many fertilization experiments have shown relationships between transplanting survival and seedling functional attributes (*e.g*. van den Driessche 1988, Villar-Salvador *et al*. 2004, Oliet *et al.* 2009). However, since plant size and nutrition covary in fertilisation studies, the specific contribution of morphology and nutrient concentration on seedling establishment cannot be disentangled. Both plant size and N concentration can independently determine seedling transplanting performance by affecting the carbon and water economy of plants (Field and Mooney 1986; Lamhamedi *et al*. 1998), the concentration of osmotically active compounds (Gebre *et al*. 1998) or the amount of N for remobilisation (Millard 1996; Silla and Escudero 2003). Although our experimental design is incomplete (due to the lack of large plants with low N concentration), it provides two results that support the hypothesis that seedling size in pine plays a more important role in seedling post-transplanting performance than N concentration. Firstly, the two small seedling types had similar survival, gas exchange, Ψ, shoot, and absolute new root growth in spite of the two-fold difference in their N concentration at transplanting. Secondly, large and small seedlings with the same tissue N concentration (*L*+ and *S*+ plants) showed remarkable differences in survival, growth, and physiological performance.

Differences between oak and pine

Whereas pine phenotypes had marked differences in physiological and transplanting performance, oak phenotypes showed much smaller differences. Distinctions between species in this study might be explained by the smaller functional differences among oak phenotypes than in pine phenotypes, which indicate lower phenotypic plasticity in the former species. Pioneer species such as *P. halepensis* tend to have higher phenotypic plasticity than late-successional species such as *Q. ilex* (Bazzaz 1979; Valladares *et al.* 2000). Our results therefore indicate that morphological and physiological differences among seedlings within a species may have different consequences for transplanting performance depending on species functional and ecological characteristics. Differences in field transplanting performance among phenotypes are expected to be greater in species with higher plasticity to nursery growing conditions and drought resistance than in species with the opposite characteristics. Future studies should further investigate this hypothesis.

Conclusions

Large pine seedlings had greater survival and growth than small seedlings, while differences among oak phenotypes were minor. Improved transplanting survival of large pines seedlings depended on the degree of environmental stress experienced by plants, with large seedlings having better performance under low and moderate stress conditions; however, no difference may occur between seedling types under severe conditions. Improved survival and growth of larger pines was attributed to higher gas exchange, root growth and N cycling, but not to water potential. Seedling size at transplanting had a greater influence on post-transplanting survival than N concentration. These findings provide a physiological basis for understanding differences in survival and growth of planted stock in Mediterranean woodland plantations and improve our understanding of the role of specific functional attributes on performance of planted seedlings.

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De la arquitectura de un soplo, la improvisación.
De la falta de reglas escritas, la imaginación.
De la belleza explícita, la determinación.
La esencia del presente, su combinación.

Óscar Godoy del Olmo

Capítulo 3

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Crecimiento radical y su interferencia con la elongación aérea en plantones de pino carrasco (*Pinus halepensis* Mill.): efectos del tamaño de planta y la concentración de nitrógeno

Este capítulo reproduce íntegramente el texto del siguiente manuscrito:

Cuesta, B., Vega, J., Villar-Salvador, P. and Rey-Benayas, J.M. Root growth and interference with shoot elongation in Aleppo pine (*Pinus halepensis* Mill.) seedlings: effects of plant size and nitrogen concentration. En revisión en Trees-Structure and Function.

Resumen

Las plantas grandes y con gran concentración de nitrógeno (N) suelen presentar mayor supervivencia y crecimiento en plantaciones forestales mediterráneas que las plantas con los atributos contrarios. Se cree que ello es debido, en parte, al desarrollo de profundos y extensos sistemas radicales en el primer tipo de plantas. Este estudio analiza el efecto del tamaño de la planta y la concentración de N en la dinámica radical en campo y su interferencia con la elongación aérea de plantones de *Pinus halepensis* Mill. Se cultivaron plantones que difirieron en tamaño y concentración de N y después se transplantaron en rizotrones. Periódicamente se midió el número, la tasa de elongación y la profundidad de las raíces, así como la elongación aérea. También se midió el potencial hídrico (Ψ) y la tasa neta de fotosíntesis. El crecimiento de las raíces nuevas tras la plantación aumentó con el tamaño de la planta. Ello se debió a una mayor proliferación de raíces con baja longitud específica más que a una mayor tasa de elongación de cada raíz individual. Ni el tamaño de la planta ni la concentración de N influyeron en la profundidad radical. Las plantas grandes presentaron menor masa de raíces por masa de hojas y menor Ψ al alba que las plantas pequeñas, mientras que no se encontraron diferencias en el Ψ medido al mediodía. La concentración de N tuvo un efecto aditivo al efecto del tamaño de la planta en el crecimiento radical pero, de forma general, su efecto fue menor que el del tamaño de los plantones. La interferencia entre el crecimiento radical y el aéreo disminuyó con el incremento del tamaño de la planta y fue independiente de la concentración de N. Concluimos que el tamaño de los plantones tuvo un efecto mayor en el crecimiento radical y en su interferencia con el crecimiento aéreo que la concentración de N. El crecimiento radical, pero no su profundidad, aumentó con el tamaño de la planta y la concentración de N en los tejidos, mientras que la interferencia entre el crecimiento radical y el aéreo disminuyó con el tamaño de los plantones de *P. halepensis*.

Palabras clave: contenido de nitrógeno, crecimiento aéreo, elongación radical, potencial hídrico, relaciones sumidero-fuente, rizotron.

Root growth and interference with shoot elongation in Aleppo pine (*Pinus halepensis* Mill.) seedlings: effects of plant size and nitrogen concentration

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Abstract

Large seedlings and plants with high nitrogen (N) concentration frequently have higher survival and growth in Mediterranean forest plantations than plants with the opposite traits. It has been hypothesized that this is, in part, due to the development of deeper and larger roots systems in the former type of seedlings. This study assessed the effect of plant size and N concentration on root growth dynamics in the field and its interference with shoot elongation in *Pinus halepensis* Mill. seedlings. We cultivated seedlings that differed in size and N concentration that were subsequently transplanted into rhizotrons. The number, elongation rate and depth of roots as well as shoot elongation rate were periodically measured. We also measured net photosynthetic rate and twig water potential (Ψ). New root growth after transplanting increased with seedling size, which was attributed to a greater proliferation of roots with low specific length rather than to a higher elongation rate of individual roots. Neither plant size nor N concentration affected root depth. Large seedlings had lower new root mass per unit of leaf mass and pre-dawn Ψ than small seedlings but no differences existed in midday Ψ. N concentration had an additive effect on plant size on root growth but its overall effect was less important than seedling size. The interference between root and shoot growth decreased with increase in seedling size and was independent on N concentration. It is concluded that seedling size had a greater effect on root growth and on its interference with shoot growth than N concentration. Root growth but not rooting depth in *P. halepensis* seedlings was increased by plant size and tissue N concentration, while root-shoot growth interference was reduced with plant size.

Key words: nitrogen content, rhizotron, root elongation, shoot growth, sink-source relations, water potential.

Introduction

Abiotic and biotic factors have a profound influence on root growth and structure (Andersen *et al.* 1986; Lopushinsky and Max 1990; Lyr 1996; Munro *et al*. 1999; Alvarez-Uria and Korner 2007). Root growth and structure also varies across plant species and are linked to species functional and ecological characteristics (Schenk and Jackson 2002; Comas and Eissenstat 2004). By contrast, fewer studies have analysed how plant functional characteristics affect root growth and structure in a given species. Present growing conditions may determine future root growth and architecture of plants. For instance, root restriction in plants cultivated in containers affects root properties after transplanting (Halter and Chanway 1993; Lindström and Rune 1999). Similarly, the capacity to produce new roots under optimal growing conditions in a greenhouse in several forests species is positively related to shoot size and N concentration (van den Driessche 1992; Villar-Salvador *et al*. 2004).

New root growth after transplanting is essential for seedling survival in forest plantations (Ritchie and Dunlap 1980; Burdett *et al*. 1983; Burdett 1990; Grossnickle 2005) as new roots allow seedlings to access soil water and mineral nutrients in the surrounding soil (Lyr and Hoffmann 1967; Grossnickle 2005; Padilla and Pugnaire 2007). Villar-Salvador (2003) suggested that higher root growth after transplanting might explain the frequently improved survival and growth of large seedlings and seedlings with high N concentration relative to seedlings with the opposite traits in Mediterranean plantations (Puértolas *et al*. 2003; Villar-Salvador *et al*. 2004; Tsakaldimi *et al*. 2005; Villar-Salvador *et al*. 2008; Luis *et al*. 2009; Oliet *et al*. 2009). To our knowledge, no study has analysed the influence of seedling size and N concentration on root growth dynamics in the field.

Plants usually have limited resources to simultaneously support their main physiological processes (growth, defence, maintenance, storage, and reproduction) (Chapin 1990; Herms and Mattson 1992; Obeso 2002). Interference in resource allocation among organs or functions increases as environmental conditions become limiting and varies with the considered organ (Thaler and Pagès 1996b; Obeso 2002). Root and shoot growth may compete for the same pool of available resources and, therefore, occur at the expense of the other. In agreement with this hypothesis, some studies have observed that roots and shoots alternate their growth through time suggesting interference between both organs (Langlois *et al*. 1983; Thaler and Pagès 1996a; Willaume and Pagès 2006). Other studies, however, have reported that root and shoot growth do not interfere each other and overlap through time (Lyr and Hoffmann 1967; Harmer 1990; Corchero-de la Torre *et al*. 2002). These differences among studies suggest different sink/source relations among species or individuals within a species. Consistently with this argument, defoliation of *Quercus pubescens* seedlings amplified the decrease in root growth concomitant with leaf expansion (Willaume and Pagès 2006). Therefore, it can be expected that individuals in a given species that have high photosynthetic capacity or high amount of stored nutrients to be remobilised will have less interference between plant functions.

The general objective of this study was to analyse the influence of seedling size and tissue N concentration on root growth and its interference with shoot growth in Aleppo pine (*Pinus halepensis* Mill.) seedlings under field conditions. Root growth dynamics for this pine in the field has been previously described (Leshem 1965; Corchero-de la Torre *et al*. 2002). We assessed the hypothesis that large seedlings and plants with high N concentration produce larger and deeper root systems and their root growth interferes less with shoot growth than the plants with the opposite attributes. To test this hypothesis, we transplanted into transparent methacrilate tubes seedlings that strongly differed in size and tissue N concentration and periodically measured root and shoot growth. We selected *P. halepensis* because it is a structural species in many types of woodland throughout the Mediterranean basin and it is commonly used in reforestation projects.

Material and methods

Seedling nursery cultivation

Seeds from an inland Spain provenance were sown in Plasnor 190/300-45 trays (45 cavities of 300 ml per tray; Plasnor, Legazpi, Spain) with unfertilized *Sphagnum* peat (Kekkilä B0, Tuusula Finland). We cultivated 270 seedlings of six phenotypes that differed in size (small, medium and large types) and N concentration (high-fertilized and low-fertilized types). To achieve differences in plant size we varied seeding date, which determined the length of the growing season. Seeds were sown on December 15, 2005 (small seedlings), March 13, 2006 (medium seedlings) and May 23, 2006 (large seedlings). After emergence, seedlings were initially fertilized weekly with a 100 ppm N fertilizer solution until September 27, 2006. Fertilization started in May 10, June 2 and August 2 for the seedlings sown in December, March and May, respectively. Subsequently, half of the seedlings were fertilized weekly with 200 ppm N until mid December 2006 (high-fertilized seedlings, N^+). The remainder half was not fertilized any more (low fertilized seedlings, N^-). Fertilization was done with a Peters Professional® 20-7-19: N-P_2O_5-K_2O fertilizer (Scotts Professional, Geldermalsen, The Netherlands).

Morphology and N concentration measurements following cultivation

Fifteen plants of each of the six phenotypes were randomly sampled at the end of the nursery cultivation for morphology and N concentration determinations. Shoots were cut at the cotyledon insertion point and root plugs were cleaned from the peat. All plant parts were then washed with tap water, rinsed in distilled water, and dried in a ventilated oven at 60 ºC for 48 h to measure their mass.

To assess tissue N concentration, plants were randomly distributed into three groups and finely ground. N concentration was determined by Kjeldahl analysis with K-$SeSO_4$-Se_2Cu_4 as catalyst in a Tecator DS-40 digestion system (FOSS Tecator, Sweden) and a SAN ++ autoanalyser (Skalar, Netherlands).

Experimental design and field measurements

The experiment was carried out in Guadalajara, central Spain (49º 39´ N, 3º 10´ W) at 650 m a.s.l. on a flat plot. The climate is Mediterranean continental with mean annual precipitation and temperature of 400 mm and 13.4 ºC, respectively. A pronounced dry and hot season occurs from June to late September and frosts in winter are frequent.

Six seedlings per nursery treatment were transplanted into transparent methacrylate tubes (here after rhizotrons) in April 2007. One seedling was planted in each rhizotron. Rhizotrons were 1 m in length, 13.5 cm in exterior diameter, and had a wall thickness of 0.5 cm. Rhizotron bottom end was closed with a perforated PVC lid, which drainage holes were covered with a mesh to prevent substrate loss. The bottom of the rhizotron was filled with gravel (*ca*. 10 cm in height) and the remainder was filled with washed and sieved sand. Subsequently, we planted the seedlings placing the root plug against the rhizotron wall. Rhizotrons were inserted into the soil to maintain roots as close as the plot soil temperature. To facilitate periodic extraction, each rhizotron was inserted into a second opaque plastic tube 1.1 m in length and 15 cm in interior diameter that was buried in the soil with a 30º inclination to force root growth against the rhizotron wall. As the top of the opaque tube and of the rhizotron protruded 10-15 cm from the ground, we wrapped an insulating sheet around the protruding portion of the opaque tube to prevent rhizotron overheating. Seedlings were thoroughly irrigated at planting and then again on April 23, May 10 and May 18 but with 200 ml per plant to simulate standard spring rainfall at the experimental site. During the rest of the experiment, seedlings were not irrigated to simulate the summer drought typical of Mediterranean climate.

When most seedlings had visible roots, we measured root elongation and depth as well as shoot height every 6-12 days from April 30 to July 11. On each date, all new roots were drawn on the same acetate sheet, which was placed over the rhizotron wall on identical position, and their length were measured after correcting for root curvatures. We counted the number of new growing roots. The roots that stopped their growth for at least 15 days were not counted. The mean root elongation rate was calculated as the mean elongation of each individual root per time unit. We also calculated the total root elongation rate of a plant as the sum of elongations of all individual roots per time unit, which estimates plant effort to expand its root system. Root depth was measured as the vertical distance from the plug bottom to the tip of each drawn root. Shoot height was measured as the distance between the cotyledon insertion point and the shoot apex, and shoot elongation rate was calculated as the differences in seedling height between two dates. We also measured the stem diameter at the cotyledon insertion point with a calliper in the first day of the field experiment to calculate the initial stem volume assuming the stem to be a cone. Stem volume was highly correlated with plant mass ($r = 0.92$, $P < 0.001$, $n = 36$) and we used it as a proxy of initial seedling size.

On the last day of the field experiment, we measured the twig water potential at predawn (Ψ_{pd}) and at midday (Ψ_{md}) in all seedlings with a pressure chamber.

In spring (late-May 2007) we measured the net photosynthesis rate (*A*) of seedling phenotypes. As the measurement of gas exchange in conifers is partially destructive, we measured *A* in a different set of individuals than those planted in the rhizotrons and that were randomly selected from the same nursery population of rhizotron plants. These seedlings were planted in February 2007 on an adjacent plot to that of the rhizotrons. *A* was measured in 12 seedlings per phenotype on June 21 and 22, 2007. Measurements were made at mid-morning and at midday (9:00 to 11:00 h and 12:00 to 14:00 h solar time, respectively) on current year shoots, using a portable gas exchange system (LCpro+ System, ADC BioScientific, Hoddesdon, UK). Photosynthetic photon flux density during measurements was > 1000 mol m^{-2} s^{-1} and air temperature ranged from 22 and 28 ºC. Seedling Ψ_{md} varied from -1.4 to -1.5 MPa.

Morphological analyses following the field experiment

At the end of the field experiment, seedlings were extracted from the rhizotrons and separated into leaves, stems and old and new roots, which were washed with tap water and dried in a ventilated oven at 60 ºC for 48 h to measure dry mass. Roots protruding out of the root plug were designated as new roots while those in the root plug were classified as old roots. Standardization of new root mass by leaf mass (*NR/L*) was used as a proxy of water balance potential of the plant. Before drying the new roots, we measured new root length according to methodology proposed in Marsh (1971). We estimated the specific root length (*SR*L) as the new root length to mass ratio.

Data analysis

Differences in seedling attributes following nursery cultivation were analysed by two-factorial ANOVA, with sowing date and fertilization as main factors. Seedling morphological attributes at the end of the field experiment, Ψ and *A* were analysed by ANCOVA, while mean and total root elongation rate and depth, and the number of roots were analysed by repeated measure ANCOVA. In theses analyses fertilization was the categorical predictor and the initial seedling size was the covariable.

The degree of interference between root and shoot growth was measured by calculating the slope of linear regressions of the standardized root rate against the shoot elongation rate. Negative slopes mean that root and shoot growth interfere, while regressions with zero or positive slopes mean no interference between roots and shoots. The effect of fertilization and plant size on the interference between root and shoot growth was analysed by ANCOVA. To assess if the slope values in each seedling type were different from zero, we carried out a t-test for single samples.

Data were checked for normality and homogeneity of variances and were transformed when necessary to correct deviations from these assumptions. In ANCOVA, we also checked the homogeneity of slopes between the covariable and dependent variables, and in all cases, treatment slopes were similar. All statistical analyses were performed with the Statistica 6.1. Package (StatSoft, Inc., Tulsa, OK, USA). For simplicity, we will represent the results of the effect of the covariable stem volume with bar diagrams, classifying plants into three size categories according to sowing date in the nursery (small, medium and large) (see Table 1).

Results

Seedling attributes following nursery cultivation

Seedling mass decreased with the delay in sowing date (F = 116.1, $P < 0.001$). Plants sown in December were 1.5 and 6.3 times larger than plants sown in March and in May, respectively, while plants sown in March were 4.3 times larger than those sown in May. By contrast, seedling mass did not differ between fertilization treatments (Table 1). Seedling shoot/root ratio was close to 1 and did not differ among sowing dates, but it was lower in N^- than in N^+ seedlings (F = 7.1, $P = 0.009$).

Fertilization increased seedling N concentration (F = 53.4, $P < 0.001$), which was on average 35% higher in N^+ than in N^- seedlings. Delay in sowing date also increased seedling N concentration (F = 15.3, $P < 0.001$); seedlings sown in May had 26 and 30% higher N concentration than those sown either in March or in December, respectively. Both sowing date (F = 81.5, $P < 0.001$) and fertilization (F = 23.5, $P < 0.001$) affected seedling N content, being highest and lowest in seedlings sown in December and May, respectively, while seedlings sown in March had intermediate values. N content was 30% higher in N^+ than in N^- seedlings.

Root and shoot growth in the field

Mean and total root elongation rates and root number increased through time (Table 2, Fig. 1). Over all dates, mean root elongation rate of individual roots was not affected by seedling size. This occurred because seedling size increased mean root elongation rate during the first weeks but it reduced it at the end of the experiment (time x initial stem volume interaction; Table 2). Fertilization reduced mean root elongation, being 25% lower in N^+ plants than in N^- plants. Total root elongation rate increased with seedling stem volume, but there were no differences between large and medium plants at the end of the study (time x initial stem volume interaction; Table 2). Fertilization did not affect total root elongation rate. The number of roots increased with initial seedling stem volume, except in the first measurement date (time x initial stem volume interaction; Table 2). Nursery fertilization increased the number of roots, N^+ and N^- plants having 20.2 ± 1.3 and 14.5 ± 1.4 roots (mean ± 1SE), respectively. Mean and maximum root depth increased through time; they ranged from 32 to 37 cm and from 51 to 61 cm, respectively, at the end of the experiment. Neither initial seedling size nor fertilization affected mean or maximum root depth (data not shown).

At the end of the experiment, both initial seedling stem volume and nursery fertilization increased new root mass and length (Table 2, Fig. 2). New root mass was 29% higher in N^+ seedlings than in N^- seedlings, and 52% and 48% lower in small seedlings than in the large and medium seedlings. New root length was 7.9 ± 0.92 and 5.6 ± 0.54 m (mean ± 1 SE) in N^+ and N^- seedlings, respectively, whereas large, medium and small seedlings had 8.0 ± 1.03, 8.1 ± 0.89 and 4.2 ± 0.52 m of new roots, respectively. Seedling stem volume and fertilization significantly enhanced growth of both shoot and old root mass.

Table 1. Morphology and N concentration content of *Pinus halepensis* seedlings that were sown at three dates and cultivated at two fertilization rates. Data are means ± one SE. n=15. Seedling types with different letter indicate significant differences at $P < 0.05$ after Tukey´s *post-hoc* test.

	December		March		May	
	N^-	N^+	N^-	N^+	N^-	N^+
Plant mass (g)	6.7 ± 0.5^a	5.9 ± 0.3^a	4.3 ± 0.3^b	4.4 ± 0.2^b	1.0 ± 0.2^c	1.0 ± 0.1^c
Shoot / Root ratio	1.1 ± 0.1^a	1.1 ± 0.1^{ab}	1.1 ± 0.05^a	1.0 ± 0.1^{ab}	1.1 ± 0.1^a	0.8 ± 0.1^b
Plant N concentration (mg g^{-1})	6 ± 0.3^d	10 ± 1.1^{bc}	7 ± 0.2^{cd}	10 ± 0.9^b	9 ± 0.6^{bcd}	14 ± 0.6^a
Plant N content (mg)	42 ± 2.5^{ab}	56 ± 3.6^a	30 ± 1.9^c	45 ± 4.7^{ab}	9 ± 2.1^d	15 ± 1.8^d

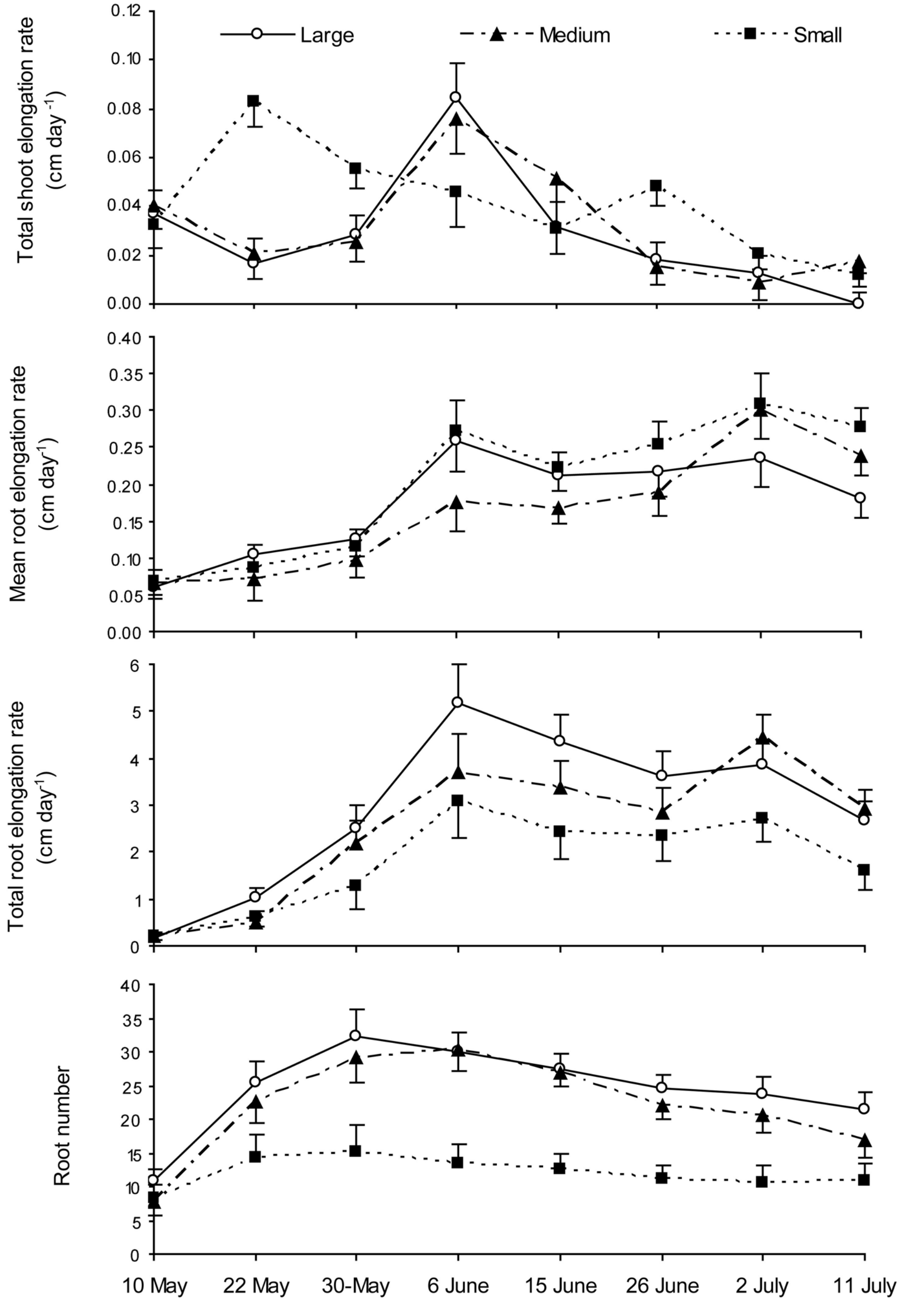

Figure 1. Variation of mean shoot elongation rate, mean and total root elongation rate and number of growing roots in *Pinus halepensis* seedlings of three size types along the experiment. Data are means ± one SE. n=6.

Increase in seedling stem volume significantly diminished *NR/L* and *SRL*, although in the latter the effect was marginal (*P* = 0.065). Medium and large seedlings had 22% and 30% lower *NR/L*, respectively, than small seedlings. Nursery fertilization did not affect either *SRL* or *NR/L.*

Mass of new roots was positively related to seedling N content (Fig. 3), plant size (r^2 = 0.53, *P* = 0.065, n = 6) and number of roots (r^2 = 0.32, *P* < 0.001, n = 36) at the end of the experiment, but not with mean root elongation rate (r^2 = 0.050, *P* = 0.19, n = 36).

Interference between root and shoot growth, seedling water potential and net photosynthetic rate

The slope of the regression of root elongation rate against shoot elongation rate was significantly and positively related to stem volume while fertilization had no effect (Table 2 and Fig. 4). Similarly, no interaction between fertilization and initial stem volume on the slope of the regression between these rates was observed (F = 0.18, *P* = 0.68). Slopes of small seedlings were significantly lower from zero (-0.26 ± 0.046 [mean ± 1SE], t = -5.63, *P* < 0.001). On the

Table 2. ANCOVA of the effects of initial stem volume (covariable), fertilization and time (sowing date) on root and shoot growth variables, twig water potential (Ψ) and net photosynthetic rate (*A*) in *Pinus halepensis* seedlings. Data are F ratios. *** *P*< 0.001, ** *P* < 0.01, * *P* < 0.05, † *P* < 0.07.

	Initial stem volume	Fertilization	Time	Initial stem volume × Time	Fertilization × Time
Shoot elongation rate	9.98***	0.074	4.24***	6.64***	0.79
Average root depth	1.76	0.01	116***	0.42	0.18
Maximum root depth	2.00	1.57	137***	1.18	1.46
Mean root elongation rate	0.32	3.49†	16.4***	2.66*	0.73
Total root elongation rate	17.51***	0.09	6.44***	4.44***	1.65
New root number	29.72***	4.91*	2.32*	6.40***	1.24
New root mass	23.11***	4.42*	—	—	—
New root mass / leaf mass	0.41	4.44*	—	—	—
New root length	11.43**	4.67*	—	—	—
New root specific length	3.51†	0.14	—	—	—
Root-shoot growth interference	19.52***	0.01	—	—	—
Old root increment	25.33***	3.99†	—	—	—
Shoot increment	25.52***	10.28**	—	—	—
Ψ	5.08*	3.64†	580***	5.61*	0.04
A	0.74	1.32	10.1**	0.35	0.27

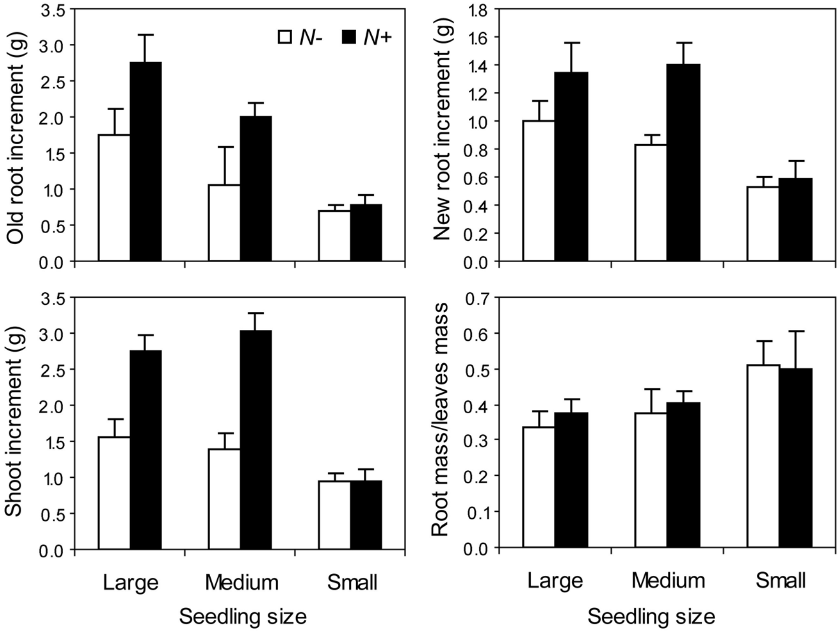

Figure 2. Old root and shoot mass increment (left) and new root production and new root mass to leaf mass ratio (right) at the end of the rhizotron experiment in *Pinus halepensis* seedlings that differed in size at transplanting and were cultivated with contrasting fertilization rate in the nursery. Data are means ± one SE. n=6.

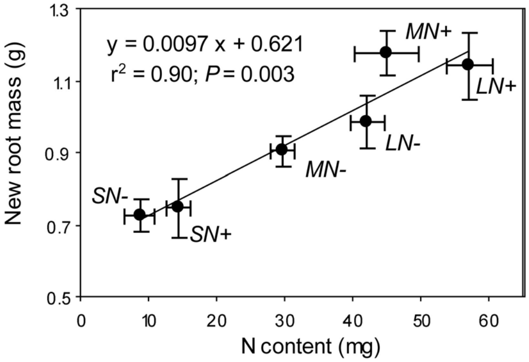

Figure 3. Relationship between new root mass and N content in *Pinus halepensis* seedlings. Data are means ± one SE. S (small seedling), M (medium seedling), L (large seedling), N^- (low fertilization) and N^+ (high fertilization).

contrary, the slope of large plants was significantly greater than zero (0.20 ± 0.089, t = 2.25, *P* = 0.045), whereas the slope of medium seedlings did not significantly differ from zero (0.12 ± 0.09, t = 1.30, *P* = 0.22).

Seedling Ψ_{pd} was higher than Ψ_{md}. Stem volume reduced Ψ_{pd} whereas it did not affect Ψ_{md} (time x initial stem volume interaction, Table 2). Ψ_{pd} for large, medium and small seedlings was -1.21 ± 0.04, -1.05 ± 0.04 and -0.96 ± 0.04 MPa, respectively, whereas Ψ_{md} was -2.53 ± -0.08, -2.53 ± 0.08 and -2.48 ± 0.08 MPa, respectively. Nursery fertilization did not affect either Ψ_{pd} or Ψ_{md}. Seedling *A* was greater at mid-morning than at midday (data not shown). *A* ranged between 11.8 µmol m^{-2} s^{-1} in small size and low fertilized seedlings and 8.4 µmol m^{-2} s^{-1} in medium size and high-fertilized seedlings but neither stem volume nor nursery fertilization significantly affected it.

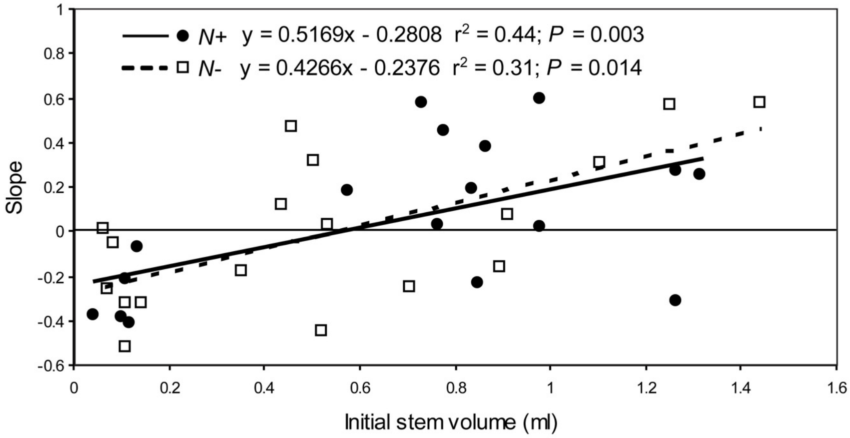

Figure 4. Relationship between the slope of the regressions of shoot against root elongation rates and seedling size in high (N^+) and low (N^-) fertilized plants. Seedling size was measured as the initial stem volume. Each point represents an individual seedling.

Discussion

Root growth and structure

In agreement with our hypothesis, large seedlings produced larger root systems than small seedlings. Similar results were found in the conifer *Juniperus thurifera* under field conditions (Martínez-Sanz 2006) and in root growth capacity tests performed in greenhouse (van den Driessche 1992; Villar-Salvador *et al*. 2004). Root systems were greater in large seedlings than in small seedlings because they produced higher number of roots rather than because individual roots had higher elongation rate. Contrary to our results, Martínez-Sanz (2006) found differences in elongation rate of individual roots among seedlings of different size in *J. thurifera*.

Seedling survival in dry ecosystems depends on the development of large and deep root systems since they determine seedling water and nutrient absorption capacity (Burdett *et al*. 1983; Grossnickle 2005; Padilla and Pugnaire 2007). Greater root systems of large seedlings could explain their frequently improved post-transplanting performance relative to small seedlings in Mediterranean plantations (Tsakaldimi *et al*. 2005; Villar-Salvador *et al*. 2008; Luis *et al*. 2009; Oliet *et al*. 2009). Contrary to our expectations, we did not find differences in root depth among seedlings phenotypes, which differs with the result found for *J. thurifera* seedlings, where roots of large seedlings grew deeper than those of small seedlings (Martínez-Sanz 2006). Absence of differences in root depth among Aleppo pine seedling types is consistent with lack of differences among seedling types in mean elongation rate of individual roots. Padilla and Pugnaire (2007) did not find any relationship between initial species size and either root elongation rate or maximum root depth in a comparison of several Mediterranean woody species. This suggests that functional processes occurring across species may not coincide with those occurring at the intraspecific level. Accordingly with our hypothesis, high-fertilized seedlings also produced greater root systems than low-fertilized plants. Nevertheless, root elongation rate of individual roots was just slightly higher in low fertilized seedlings than in high-fertilized seedlings, which can explain the similar total root elongation rates between fertilization regimes.

Specific root length depends on root thickness and/or density and varies widely among species, although it usually has low variation in response to environmental conditions such as temperature or nutrient availability (George *et al*. 1997; Reich *et al*. 1998; Pregitzer *et al*. 2002; Alvarez-Uria and Korner 2007). We found that at the intraespecific level, small seedlings tended to have higher *SRL* than large seedlings; this suggests that the former produced thinner and/or less dense roots, which have lower construction and maintenance cost than low *SRL* roots (Pregitzer *et al*. 2002). Higher *SRL* in larger seedlings also contributed to their greater root mass relative to smaller seedlings.

NR/L provides an idea of the potential balance between the water transpiration and water uptake capacity in a plant. High *NR/L* may confer greater capacity of plants to maintain high water potential under drought conditions. Large seedlings in our study were similarly water balanced than small seedlings in spite of having lower *NR/L*. This argument is supported by the lack of differences in midday Ψ among seedlings of different size. Higher root density (mass of new roots per soil volume unit) and enhanced root hydraulic conductance in larger plants (Wan *et al*. 1996; Chirino *et al*, 2008) in comparison to smaller plants could explain why large seedlings, in spite of having lower *NL/R*, had similar water potential at midday than small seedlings. Small seedlings had lower pre-dawn Ψ than large seedlings, which indicates that small plants can get rehydrated more rapidly at night than large seedlings. This response, however, might be a consequence of their smaller shoot rather than improved water balance.

Seedling size had greater effect on root growth than nursery fertilization, as much variables were affected by seedling size than by fertilization, and when both variables were significant, seedling size was more significant than fertilization (Table 2). Differences in size among seedling phenotypes were higher than differences in N concentration, explaining the greater effect of seedling size on root growth. As root growth in *P. halepensis* and other conifers strongly relies on current photosynthesis (van den Driessche 1987; Moreno 2003), an increase in root growth with seedling size might be attributed to higher photosynthesis in larger plants than in small plants. Greater photosynthesis in larger plants in our study was due to greater foliage, but not to greater *A*. Remobilisation of stored N also supports growth of new organs and this mechanism is usually dependant on the amount of stored N (Malik and Timmer 1996; Millard 1996; Dyckmans and Flessa 2001; Salifu and Timmer 2003). In our study, large plants had also higher N content than small seedlings. Nursery fertilization, which increased plant N concentration without promoting growth, had an additive effect over the effect of plant size on root growth (see Fig. 3). N concentration can stimulate growth by increasing the amount of remobilisable N and/or enhancing photosynthetic rate (Field and Mooney 1986). As fertilization treatments did not differ in *A*, the positive effect of tissue N concentration on root growth was probably related to higher availability of remobilisable N. In agreement with this argument, the root mass-N content relationship was tighter than the root mass-plant mass relationship (see Puértolas *et al*. 2003).

Interference between root and shoot growth

As resources become limited, investment of resources to root growth can reduce shoot growth or reproduction and *vice versa* (Bloom *et al*. 1985; Chapin 1990). Interference between root and shoot growth differs among species and organs (Lyr and Hoffmann 1967; Riedacker 1976; Reich *et al*. 1980; Harris *et al*. 1995; Thaler and Pagès, 1996a). However, we demonstrate that root and shoot growth interference also varies among individuals of contrasted size in Aleppo pine. Accordingly to our hypothesis, root and shoot growth did not interfere in large seedlings but it did in the small ones. Willaume and Pagès (2006) found that reduction of photosynthesis capacity by defoliation increased the interference between root and shoot growth in *Quercus pubescens*, highlighting the importance of carbohydrate sink/source relations in this interference. The absence of N fertilization effects indicates no additive effects of N concentration over the plant size effects on the interference between root growth and shoot growth. This suggests that differences in photosynthetic capacity due to distinct amount of foliage N rather than differences in stored N is the main mechanism underlying the differences in shoot-root growth interference.

Conclusions

Large seedlings developed denser but not deeper root systems than small seedlings due to greater proliferation of new roots with lower specific root length in the former. Seedling size had greater influence on root growth than N concentration, which had an additive effect over plant size. Root and shoot growth interfered in small seedlings, whereas it did not occur in large seedlings. These findings provide insights for understanding root growth differences and transplanting performance among Aleppo pine stock-types in Mediterranean woodland plantations.

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Mira tu entorno y aprende de tus amigas las "plantas":
ante la adversidad se fuerte.
Algunas veces tendrás que facilitar y competir
por el desarrollo de la vida.

Liliana Tovar González

Capítulo 4

Capítulo 4

La facilitación de encinas en matorrales mediterráneos se explica por interacciones directas e indirectas mediadas por hierbas

Este capítulo reproduce íntegramente el texto del siguiente manuscrito:

Cuesta, B., Villar-Salvador, P., Puértolas, J., Rey Benayas, J.M., and Michalet, R. En prensa. Facilitation of oaks in Mediterranean shrubland is explained by both direct and indirect interactions mediated by herbs. Journal of Ecology.

Resumen

Las interacciones de competencia y facilitación determinan el funcionamiento de la comunidad de plantas. La importancia de estas interacciones varía a lo largo de gradientes de estrés y depende de las especies consideradas, de su etapa en el ciclo de vida y de las interacciones con otros vecinos. Mientras que la competencia y la facilitación directa han sido ampliamente estudiadas en ambientes secos, la facilitación indirecta entre plantas ha recibido poca atención. Investigamos la importancia relativa de la facilitación directa e indirecta mediada por la supresión de hierbas en un matorral mediterráneo en dos años consecutivos. La planta nodriza fue la leguminosa arbustiva *Retama sphaerocarpa* (retama) y la especie facilitada fue la encina (*Quercus ilex*), una especie tardía en la sucesión. También estudiamos si el resultado de la facilitación depende del tamaño de los plantones de encina. Se llevó a cabo un experimento factorial en condiciones de campo para evaluar el efecto de 1) la posición de los plantones de encina con respecto al dosel del arbusto (bajo el arbusto o claro), 2) la competencia herbácea (presencia o ausencia), y 3) el tamaño del plantón de encina. El primer año fue pluviométricamente normal mientras que el segundo año tuvo una primavera mucho más húmeda y un verano más seco que el primero. En ambos años, el arbusto nodriza redujo la mortalidad de los plantones de encina mientras que la hierba la incrementó. En el año pluviométricamente normal la mortalidad de encinas bajo los arbustos no se vio afectada por la presencia de hierbas mientras que en los claros fue significativamente mayor en presencia de éstas. Ello indica que el arbusto nodriza facilitó indirectamente a los plantones de encina suprimiendo la capacidad competitiva de las hierbas. Por el contrario, la facilitación fue predominantemente directa durante el año de primavera húmeda y verano seco, ya que las hierbas redujeron la supervivencia de los plantones de forma similar bajo el arbusto nodriza y en los claros. El arbusto nodriza facilitó directamente a las encinas reduciendo la radiación y la temperatura y, por tanto, la foto-inhibición y estrés hídrico de los plantones. La mejora de las condiciones ambientales por la retama benefició a los plantones pequeños pero no a los grandes ya que el arbusto nodriza redujo la mortalidad de los plantones pequeños en relación con los claros pero esto no ocurrió en el caso de los plantones grandes. Ello indica que los individuos dentro de una población de plantones pueden tener una respuesta diferente a la facilitación. Concluimos que tanto la facilitación directa como la facilitación indirecta son mecanismos importantes para la regeneración de encinas en los retamares y su importancia parece variar con las condiciones climáticas. La facilitación indirecta por supresión de la competencia herbácea es importante en años de primaveras secas cuando la competencia entre el arbusto nodriza y las hierbas es grande, mientras que la facilitación directa por amortiguación de la severidad microclimática aumenta con la aridez estival.

Palabras clave: competencia herbácea, fluorescencia de la clorofila, potencial hídrico, *Quercus ilex*, *Retama sphaerocarpa*, tamaño del plantón.

Facilitation of oaks in Mediterranean shrubland is explained by both direct and indirect interactions mediated by herbs

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Abstract

Competitive and facilitative interactions shape plant communities. Their importance varies along stress gradients and depends on the species, life cycle stages, and interactions with other neighbours. Whereas a number of studies have addressed competition and direct facilitation among plants in dry ecosystems, indirect facilitation among plants has received little attention. We investigated the relative importance of direct facilitation and indirect facilitation mediated by herb suppression in a Mediterranean shrubland in two consecutive years. The nurse plant was the leguminous shrub *Retama sphaerocarpa* and the target species was the late-successional oak *Quercus ilex*. We also studied whether the facilitation outcome depended on the size of the facilitated oak seedlings. A factorial field experiment was carried out to test 1) position of oak seedling with respect to shrub canopy (under shrubs or in gaps), 2) herb competition (presence or absence of herbs), and 3) oak seedling size. The first year was an average rainfall year while the second year had a much more humid spring and a dryer summer than the first year. In both years, nurse shrubs reduced oak seedling mortality whereas herbs increased it. In the average rainfall year, oak mortality under shrubs was unaffected by herb presence whereas in gaps it was significantly higher in presence of herbs. This showed that the nurse shrub indirectly facilitated the oak seedlings by suppressing the competitive capacity of herbs. Conversely, facilitation was predominately direct during the humid spring and dry summer year since herbs hindered seedling survival similarly under the nurse shrub and in gaps. The nurse shrub directly facilitated the oaks by reducing irradiance and leaf temperature, and therefore, seedling photo-inhibition and water stress. Improvement of environmental conditions by *Retama* benefited smaller seedlings but not larger seedlings since the nurse shrub reduced mortality of smaller seedlings relative to that in gaps but this effect was not observed for larger seedlings. This indicates that individuals within a seedling population may not have the same response to facilitation. We concluded that both indirect and direct facilitation are important mechanisms for oak regeneration in *Retama* shrubland and their importance seems to vary with climatic conditions. Indirect facilitation by release of herb competition under nurse shrubs is important in years of dry springs when competition between nurse shrubs and herbs is high, whereas direct facilitation mediated by microclimate amelioration increases with summer aridity.

Key words: chlorophyll fluorescence, herb competition, *Quercus ilex*, *Retama sphaerocarpa*, seedling size, water potential

Introduction

Biotic interactions are major drivers of the structure and dynamics of plant communities (Lortie *et al*. 2004). Competition has traditionally been considered as the major interaction that structures plant communities (Grime 1974). However, in the last decade, an increasing number of studies have also emphasised the importance of facilitative or positive interactions (Bertness and Callaway 1994; Callaway 1995; Brooker *et al*. 2008). Positive and negative interactions among organisms act simultaneously, and the net balance of these interactions determines the community organization and composition (Callaway and Walker 1997; Holmgren *et al.* 1997; Brooker *et al*. 2008). The balance of plant-plant interactions is context-dependent, varying in response to abiotic stress, disturbance, life cycle stage, species identity, and interactions with other neighbours (Callaway 2007).

Facilitation can be direct, *i.e.* one species increases the performance of a second species by ameliorating the abiotic environment (Callaway 1995; Pugnaire *et al.* 1996a; Pugnaire *et al*. 2004). Facilitation may also be indirect, when a third species mediates interactions between the nurse species and the target species. Thus, some plants protect other plants from herbivores, attract pollinators, concentrate propagules of other species, or enhance mycorrhizae and soil microbial activity (Callaway 1995, 2007). The occurrence of a third plant species may also convert the competition between two species into indirect facilitation via suppression of a shared competitor (Miller 1994; Levine 1999; Callaway and Pennings 2000). Levine (1999) predicted that indirect facilitation mediated by a third competitor would mainly occur in a system in which the three species compete for different resources or use different mechanisms to acquire them. This argument is supported by previous studies (Siemann and Rogers 2003; Kunstler *et al*. 2006).

In contrast to direct facilitation and competition, indirect facilitation has received less attention. Most studies assessing indirect facilitation have focused on interactions between different trophic levels, such as those analysing defence against herbivory (Boulant *et al*. 2008; Gómez-Aparicio *et al.* 2008; Anthelme and Michalet 2009) or concentration of progagules of other species (Verdú and García-Fayos 2003; Aerts *et al.* 2006). Experimental field studies investigating indirect facilitation within the same trophic level are much less frequent (Brooker *et al*. 2008). Most studies of indirect facilitation among plants have been performed in productive or moderately productive environments; whereas some of them documented indirect facilitation (Levine 1999; Callaway and Pennings 2000; Siemann and Rogers 2003; Kunstler *et al*. 2006), others failed to detect this process (Pagès and Michalet 2003; Pagès *et al.* 2003). To our knowledge, no experimental field study to date has explored the importance of indirect facilitation in semi-arid or arid systems.

Direct facilitation is a recognised recruitment mechanism for plants in Mediterranean environments (Verdú and García-Fayos 2003; Gómez-Aparicio *et al*. 2005a), but the importance of indirect facilitation remains to be assessed in this system. *Retama sphaerocarpa* (*Retama*) is one of the most studied Mediterranean nurse species. It promotes the development of a diverse herbaceous community due to changes in microclimate and soil fertility under its canopy (Pugnaire *et* *al.* 1996b; Pugnaire *et al.* 2004). Although herbs compete with the seedlings of woody species and impair woodland regeneration (Nambiar and Zed 1980; Rey Benayas *et al*. 2005), mid and late-succession woody species such as *Quercus ilex* (holm oak) develop under *Retama* (Tovar 2009). Several studies have demonstrated the direct facilitative effect of *Retama* on the plant community developed under its canopy, but no study has assessed its potential indirect facilitative effect (Pugnaire *et al*. 1996a; Pugnaire *et al.* 1996b; Rodríguez-Echeverría and Pérez-Fernández 2003; Pugnaire *et al.* 2004).

Evidences that the response to facilitation and that the facilitating ability of nurse species are species-specific are being increasingly reported (Gómez-Aparicio *et al.* 2004; Liancourt *et al.* 2005; Padilla and Pugnaire 2009). However, to our knowledge, no study has tested whether the response to facilitation varies among individuals of the same species. Individuals within the same species may have distinct functional traits that may determine their performance. For example, holm oak seedlings produced by different mother trees exhibit significant size differences that influence their performance (Leiva and Fernández-Alés 1998). Seedling size can affect survival, with large seedlings having a greater chance of survival than small seedlings under stressful conditions (Cook 1980; Leishman and Westoby 1994).

In the present study, we addressed the following questions: 1) is indirect facilitation mediated by herbs, in addition to direct facilitation, an important mechanism for *Q. ilex* regeneration in *Retama* shrubland? 2) Does the response of oak seedlings to facilitation depend on their size? To answer these questions, we conducted a factorial field experiment in two consecutive years. We measured the performance of holm oak seedlings of contrasting size planted under *Retama* canopy and in gaps, as well as in presence and absence of herbs.

Material and methods

Study site and species studied

The study site is located in La Mancha, central Spain (38º 38´ N, 3º 28´ W), at an altitude of 714 m a.s.l. The climate is continental Mediterranean, with a mean annual precipitation of 370 mm and a mean annual temperature of 15.2 ºC. Summer is hot and dry and lasts for three to five months, while winter is cold with frequent frosts. The soil is a poorly developed *Inceptisol* with relatively high clay and quartzite content. The experiment was performed on a flat abandoned wheat cropland that was planted with *R. sphaerocarpa*, *Pinus pinea*, and *Q. ilex* in 2000. Each of these three species had the same density, and individuals were randomly planted in a 3 × 4 m regular pattern. At the start of the experiment, pines and oaks were more than 2 m and 1m-high, respectively, while *Retama* shrubs were 2 m-high approximately. The understorey vegetation was composed of an herbaceous community, which emerges in the autumn, flowers in spring, and senesces during early summer. Annuals such as *Avena barbata*, *Bromus* spp., *Hordeum murinum, Lolim rigidum, Taeniathezum caput-medusa,* and *Rapistrum rugosum* and several *Trifolium* species dominated this community.

The nurse species *R. sphaerocarpa* is a leguminous, broom-like deep-rooted shrub that is native to the Iberian Peninsula and northwest Africa, where it forms shrublands that have been managed for sheep grazing and hunting. In the last decades, this shrub has colonised large areas of abandoned cropland, and it has been used for subsidised plantation programs. The target benefactor species was *Q. ilex* (holm oak), an evergreen late-successional oak tree native to the western Mediterranean Basin. It comprises most of the community biomass in areas where it thrives. However, holm oak seedlings display low survival rates in forest plantations compared with other Mediterranean forest species (Baeza *et al*. 1991).

Experimental design

A factorial field experiment was performed with three factors: 1) position with respect to the *Retama* canopy (under or outside), 2) herb abundance (presence or absence) and 3) oak seedling size. The experiment was conducted in 2006 and 2007. In 2006, the rainfall and mean temperature during the experimental period (February-September) were 255 mm and 19.2 ºC, respectively, while in 2007 they were 352 mm and 17.7 ºC, respectively. The mean historical rainfall (over a 70-year period) and temperature (over a 55-year period) during the same period were 256 mm and 17.5 ºC, respectively. Thus, 2006 can be considered as an average rainfall year and 2007 as a humid year. Differences between years mainly occurred in spring (February to May), which was much more humid in 2007 than in 2006 (150 and 267 mm in 2006 and 2007, respectively). However, summer (June to September) was more humid in 2006 than in 2007 (105 and 85 mm in 2006 and 2007, respectively).

We randomly selected 30 *Retama* shrubs with an average canopy height and diameter of 2 and 2.3 m, respectively, and 30 gaps located 1.5-2 meters outside of the shrub canopies, where the ground was not shaded during most of the day. Each *Retama* was separated from its neighbour 3-4 m. The distribution of the experimental *Retama* shrubs and gaps was not paired, *i.e*. a particular *Retama* was not associated with a particular gap in the field. In each position (under shrub and gap), we distinguished two herb abundance levels (presence or absence). In the microsites with absence of herbs, herbaceous vegetation was removed with herbicide in an area of 0.55-0.6 m^2 (Oxifluorfen 24%, Iteike, TradeCorp, Spain) at the beginning of the experiment in both years. Finally, in each of these four microsites, oak seedlings were planted in 30 cm-deep holes made with a mechanical auger, minimising the disturbance of the herbaceous community at the herb presence microsites. Planting was performed in February in both years. The experimental plot was fenced to exclude medium and large herbivores.

We planted seedlings that largely varied in size in both years. In 2006, to promote seedling size differences, we varied 1) the seeding sown date in the nursery, which affected emergence time and, therefore, the length of the growing period (14 months *vs.* 11 months), and 2) we used different nitrogen fertilization rates. In 2007, we did not apply any cultivation treatment and randomly selected the plants from a standard nursery crop that had a high variation in seedling size. In 2006, the 10th and 90th percentiles of plant height were 9 and 22 cm, respectively, while in 2007 they were 12 and 28 cm, respectively. The seedlings were cultivated following standard nursery cultivation methods (Villar-Salvador *et al*. 2004). The acorns used to grow seedlings were of the same provenance in both years.

Microclimate, soil and herb community characteristics

To analyse the effect of the nurse shrub on the environmental conditions under its canopy and on the characteristics of the herb community, we measured several abiotic and biotic variables under the*Retama* canopy and in the gaps. Incident photosynthetic photon flux density at seedlings' mid-height was measured using a quantum photo/radiometer (HD 9021, Delta OHM, Casselle Di Selvazzano, Italy) for all of the planted seedlings. Measurements were done in May of both years on clear days between 12:00 and 14:00 h solar time. The relative humidity and the temperature in the air and leaf temperature were measured in spring of 2006 under the canopy of nine shrubs and in nine gaps. The former were measured in both presence and absence of herbs with a thermo-hygrometer (HI 9065, HANNA Instruments, Eibar, Spain). The leaf temperature was only measured in the microsites with herbs using a thermocouple inserted under a dry average-sized oak leaf, which was held horizontally during the measurements.

Soil samples excluding litter and stones were collected 5-10 cm-deep under the canopy of nine shrubs and in nine gaps for nutrient analyses. After air-drying, the samples were sieved through a 2 mm sieve and finely ground. The organic matter was analysed using the method proposed by Walkley and Black (1934). The total N concentration was determined by Kjeldahl analysis with $SeSO_4$-K_2SO_4 as the catalyst, while the K concentration was measured with an Optic PLASMA ICP (Perkin-Elmer, model 4300 DV, USA), according to MAPA (1986). The soil pH was determined for a 1:2.5 mass to volume soil and water suspension. The soil temperature at a 5 cm depth was recorded with a digital thermometer in May 2006 in both presence and absence of herbs in a set of nine *Retama* shrubs and nine gaps. Finally, the soil water content in a 0-30 cm soil profile was measured in all planted seedlings in late spring (May 25th in 2006 and May 30th in 2007) and in mid-summer (July 18th in 2006 and July 15th in 2007) using Time Domain Reflectometry (TDR, Tektronix, Beauverton, CO, USA) according to Cassel *et al.* (1994). Two stainless-steel rods 35 cm in length were inserted one month after planting at a 5-10 cm distance from plants.

In late May of both years, when flowering of the herbaceous community was ending, we visually recorded the percentage of herb cover around each oak seedling, as well as the specific composition and abundance of herbs under nine nurse shrubs and in nine gaps using a 50 x 50 cm quadrat. Herb species were classified as graminoids and non-graminoids. In early June, when the herbaceous community started to wither, the above-ground herb biomass contained in a 50 x 50 cm quadrat was measured in nine *Retama* shrubs and nine gaps after drying at 50 ºC for two days. The *Retama* shrubs and gaps used to measure the air relative humidity and air, leaf and soil temperature were randomly selected among the shrubs used to plant the oaks, whereas the *Retama* shrubs and gaps used to determine the soil nutrient concentration and herb mass were randomly selected among shrubs and gaps that were not used to plant the oaks.

Holm oak seedling performance

The mortality of *Q. ilex* seedlings was recorded at the end of spring (last week of May) and at the end of the summer (last week of September) in both years. We measured the spring (from February to June), summer (from June to September) and all first growing season (from February to September) seedling mortality. Seedling growth was measured as the stem volume increase and calculated as the difference between the seedling stem volume at the end of the summer and at the planting date. Stem volume was calculated from the height and diameter of the stem, assuming that the stem of the plant was a cone. The diameter was measured immediately above the cotyledon insertion points. We measured the maximum photochemical efficiency of photo-system II (F_v/F_m) and the leaf water potential (Ψ) in mid summer of both years (July 7th in 2006 and July 2nd in 2007) in 3-8 seedlings per treatment to assess the physiological performance of oak seedlings. F_v/F_m is a good indicator of plant photo-inhibition caused by stress factors such as drought, high radiation and frost (Maxwell and Johnson 2000), whereas Ψ is a measure of the water status of plants (Flexas *et al*. 2004). F_v/F_m was measured with a portable fluorometer (Hansatech Instruments, Norfolk, England) at predawn and midday (between 12:00 and 14:00 h solar time) after 30 minutes of dark adaptation. Ψ was determined at midday using a pressure chamber; it was measured at midday because it usually reflects the maximum water stress experienced by the plant within a day. We only measured Ψ once a day to prevent distorting the water and carbon economy of seedlings.

Data analysis

Differences among microsites in 2006 with regard to air relative humidity and leaf temperature were analysed with two-way ANOVA, where the main factors were position (under *Retama* canopy *vs*. gaps) and herb abundance (absence *vs*. presence of herbs). The differences between the nurse shrub and the gaps, both in presence of herbs, in soil nutrient concentration and pH, and leaf temperature in 2006 were analysed with Student's t-test. Differences in irradiance among treatments were analysed with ANOVA where the main factors were year (2006 *vs*. 2007), position and herb abundance. Soil water content was analysed with repeated measures ANOVA where between-effects were year and position and the within-effect was season (spring *vs*. summer). Total herbaceous mass was analysed with ANOVA, where the main factors were year and position. Differences in the floristic composition of the herbaceous community were analysed with semi-parametric MANOVA, where dependent variables were the cover of every herb species. Graminoid and non-graminoid covers were analysed with ANOVA in which the main factors were year, position, and herb guild (graminoid *vs.* non-graminoid).

Seedling survival was analysed using a generalised linear model with a binomial distribution and a logit link function that included an herb competition surrogate (see below) and seedling stem volume at the planting date as co-variables. Seedling stem volume was highly correlated with plant mass ($r = 0.92$, $P < 0.001$, $n = 30$). Growth and F_v/F_m in both years were analysed using ANCOVA. The factors in these analyses were year and position; as in the previous analysis, the surrogate of herb competition and seedling stem volume at the planting date were co-variables. We used an herb competition co-variable instead of a qualitative factor (absence *vs.* presence of herbs) in these analyses because the herb community under *Retama* and in gaps had a different floristic composition and aboveground biomass (Table 1), which can lead to a distinct competition capacity of herbs at each position. The herb competition co-variable values were calculated for each planted seedling in the spring (late May) and summer (mid July) using the factor scores obtained from a Principal Component Analysis on herb cover, soil volumetric water content and photon flux density around the seedlings. The values of soil water content and irradiance were relative values from field measurements taken under each *Retama* and in each gap; they were calculated as the relative reduction in soil humidity and irradiance in the microsite with herbs with respect to the adjacent microsite without herbs. This removes the effect of the nurse shrub. As the Ψ of many of the plants exceeded the measurement limit of the pressure chamber (6.8 MPa) in both years, data were grouped into Ψ classes, and the effect of year, position, herb competition and initial seedling volume was analysed by an ordinal multinomial lineal model with a generalised logit link.

Data were checked for normality and homogeneity of variance, and were transformed when necessary to correct deviations from these assumptions. All statistical analyses were performed with the Statistica 6.0. Package (StatSoft, Inc., Tulsa, OK, USA), except the semi-parametric MANOVA that was performed with R.2.8 (R Development Core Team, 2008).

Results

Microclimate, soil and herb community characteristics

Herbs and the *Retama* canopy reduced irradiance similarly in both years (statistical results not shown). The *Retama* canopy reduced irradiance by 33%. The reduction of irradiance by herbs was greater in gaps than under the *Retama* canopy (position × herb competition interaction, Table 1). The *Retama* canopy with herbs was the microsite with the lowest irradiance. Leaf and soil temperature were significantly lower under the *Retama* canopy than in gaps. Neither soil chemical properties nor relative humidity or temperature of the air significantly differed among the four microsites (Table 1).

Overall microsites, soil water content in spring was 14% higher in 2007 than in 2006, while in summer it was 16% lower in 2007 than in 2006 (year × season interaction; $F_{1,302} = 45.63$, $P < 0.001$) (Fig. 1). Soil water content was lower under the *Retama* canopy than in gaps, but this difference was only observed in spring and not in summer (position × season interaction; $F_{1,302} = 5.87$, $P = 0.016$). Herbs reduced soil water content both under the *Retama* canopy and in gaps, although this reduction was statistically significant in spring but not in summer (herb competition × season interaction; $F_{1,302} = 8.61$, $P = 0.004$). Soil water content under the *Retama* canopy with herbs in spring was 20 % lower in 2006 than in 2007.

Herb mass was lower in 2006 than in 2007 ($F_{1,57} = 12.22$, $P = 0.01$) and under *Retama* canopy than in gaps ($F_{1,57} = 11.78$, $P = 0.02$) (Table 1). However, the relative reduction of herb mass under *Retama* compared to gaps was higher in 2006 than in 2007 (38% *vs.* 18%, respectively). The composition of the herbaceous community under the *Retama* differed from the composition detected in gaps in 2006 but not in 2007 (year × position interaction; $F_{1,\ 35} = 4.54$, $P < 0.001$). In 2006, the non-graminoid cover was significantly lower under the *Retama* than in gaps, whereas the graminoid cover did not differ significantly between positions. By contrast, the cover of

Table 1. Microclimate features, soil properties, herb biomass and the cover of graminoids and non-graminoids herbs in the four studied microsites. Microclimatic and soil variables were measured in 2006. Values are mean ± one SE. Means with different superscript letters are significantly different at P < 0.05 after a Tuckey *post-hoc* test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. † Statistical data are reported in the text.

Variables	Microsites				F		
	Under Retama		Gap		Position (P)	Herb Competition (HC)	P × CH
	Without herbs	With Herbs	Without herbs	With herbs			
Irradiance (%)	38 ± 1.2^{c}	28 ± 0.9^{d}	100 ± 0.0^{a}	46 ± 1.5^{b}	430^{***}	225^{***}	19.9^{***}
Air relative humidity (%)	10.1±0.8	9.8±0.7	10.0± 1.0	9.8±0.8	0.04	0.08	0.007
Air temperature (Cº)	36.2±0.5	36.0±0.5	36.1±0.5	37.4±0.5	1.4	1.25	0.02
Soil temperature (Cº)	30.4 ± 1.1^{b}	28.2 ± 0.5^{b}	41.4 ± 0.6^{a}	39.5 ± 1.0^{a}	179.7^{***}	5.82^{**}	0.01
Leaf temperature (ºC)		43.1 ± 2.6^{b}		49.6 ± 1.4^{a}		$t = 6.82^{***}$	
Soil organic matter (%)		1.1±0.07		1.1±0.05		t = -0.40	
Soil N (mg kg $^{-1}$)		0.8±0.05		0.8±0.1		t = -0.98	
Soil K (mg kg $^{-1}$)		193±41.2		186±46.5		t = -0.32	
Soil pH		5.7±0.2		5.8±0.5		t = 0.49	
Herb mass in 2006 (g)		42 ± 3^{b}		67 ± 4^{a}		†	
Herb mass in 2007 (g)		67±7		81±6		†	
Graminoid cover in 2006 (%)		31±5		27±5		†	
Non-graminoid cover in 2006 (%)		4 ± 2.0^{b}		49 ± 7^{a}		†	
Graminoid cover in 2007 (%)		19±6		20±4		†	
Non-graminoid cover in 2007 (%)		19±4.9		26±6		†	

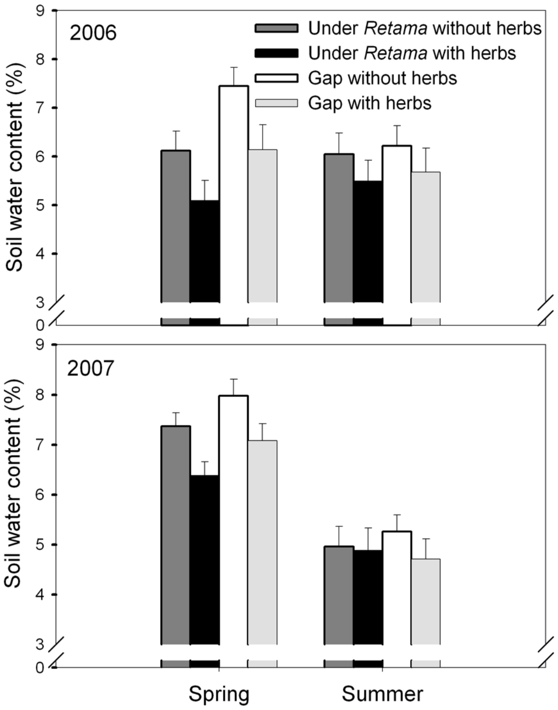

Figure 1. Soil water content at the four microsites in 2006 (above) and 2007 (below). Data represent mean values ± one SE.

both groups of herbs was similar under the *Retama* and in gaps in 2007 (year × position × herb guild interaction; $F_{1,\ 64} = 9.02$, $P = 0.004$).

Holm oak seedling performance

Seedling mortality of the introduced plants at the end of the experiment was 83.3% in 2006 and 76.9% in 2007, but these differences were not statistically significant (Table 2). Significant mortality occurred in the spring of 2006, but not in the spring of 2007 (Fig. 2). Seedling mortality was lower under the *Retama* canopy than in the gaps and in the absence of herbs than in their presence in both years (Table 2 and Fig. 2). However, the negative effect of the herbaceous community differed between years. Herbs increased seedling mortality in gaps but not under *Retama* canopy in 2006 and this effect was already apparent in spring. In contrast, herbs similarly increased seedling mortality at both positions in 2007 and it occurred later, in summer (year × herb competition × position interaction, Table 2). Plant size negatively affected oak seedling mortality (Fig. 3); however, this effect was only observed in gaps but not under the *Retama* canopy (position × seedling size interaction in Table 2). None factor influenced seedling growth (data not shown).

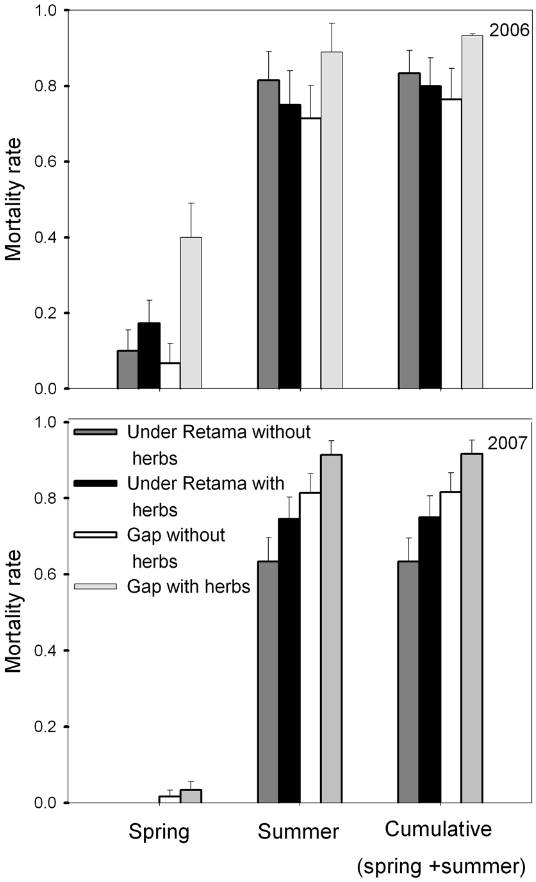

Figure 2. Mortality rate of *Quercus ilex* seedlings at the four microsites during spring, summer and the whole first growing season in 2006 (above) and 2007 (below). Data represent means ± one SE.

Table 2. Results of the ANCOVA models used to test the effects of year, position, herb competition, and seedling size on *Q. ilex* seedling mortality. The year × position, year × herb competition × position, year ×seedling size × position, and year × position × herb competition × seedling size interactions were not analyzed in spring because there was no mortality under *Retama* canopy in May 2007.

Factor	Spring		Summer		Cumulative (spring + summer)	
	χ^2	*P*	χ^2	*P*	χ^2	*P*
Year (Y)	57.8	< 0.001	0.13	0.71	2.02	0.15
Herb competition (HC)	26.5	< 0.001	3.62	0.057	6.95	0.008
Seedling size (SS)	1.72	0.19	0.005	0.94	0.03	0.86
Position (P)	9.60	0.002	11.35	< 0.001	14.6	< 0.001
Y × HC	0.51	0.47	1.35	0.24	0.309	0.58
Y × SS	0.27	0.60	0.003	0.96	0.005	0.95
Y × P			1.03	0.31	0.62	0.43
HC × SS	0.34	0.58	0.0004	0.98	0.016	0.90
HC × P	0.40	0.53	4.04	0.044	4.82	0.028
SS × P	0.09	0.77	3.79	0.051	3.85	0.049
Y × HC × SS	0.59	0.44	0.87	0.35	1.16	0.28
Y × HC × P			3.6	0.057	3.96	0.047
Y × SS × P			0.56	0.45	0.52	0.47
HC × SS × P	0.19	0.66	0.002	0.96	0.0003	0.99
Y × HC × SS × P			2.9	0.087	2.45	0.12

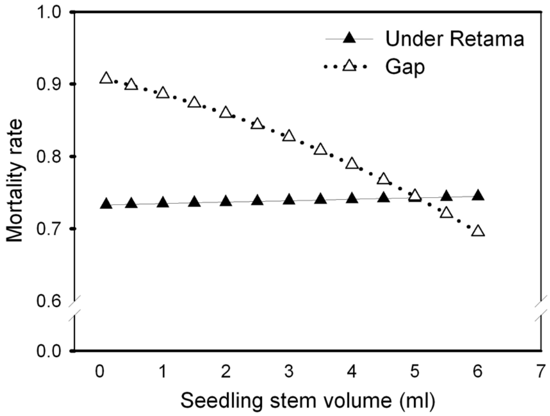

Fig. 3. Mortality probability of *Q. ilex* seedling as a function of seedling size under the *Retama* canopy and in gaps across the two years. Lines are the fitted logistic regressions. α and β parameters of the logistic regression were 2.2773 and -0.227 for the gap and 1.0087 and 0.0101 under the *Retama*.

F_v/F_m was higher under the *Retama* canopy than in gaps in both years ($F_{1,76}$ = 5.03, P = 0.027; Fig. 4) and in 2006 than in 2007 ($F_{1,76}$ = 5.28, P = 0.024). F_v/F_m tended to be higher at midday than at predawn, although this was only observed in 2007 but not in 2006 (measurement time × year interaction, $F_{1,76}$ = 3.79 P = 0.052). Neither herb competition ($F_{1,76}$ = 0.02, P = 0.89) nor seedling size ($F_{1,76}$= 0.10, P = 0.75) affected F_v/F_m.

Ψ was higher in 2006 than in 2007 (χ^2= 28.6, P < 0.001). The frequency of oak seedlings with higher (less negative) Ψ was greater under the *Retama* canopy than in gaps. This difference was more apparent in 2006 than in

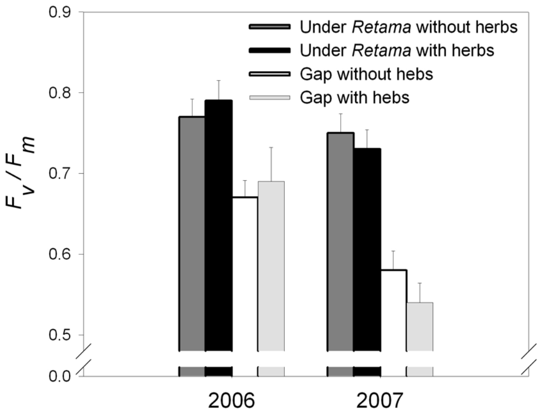

Figure 4. Photochemical efficiency (F_v / F_m) of photosystem II of *Q. ilex* seedlings planted at the four microsites in 2006 and 2007. Data represent the average between morning and midday data. Values are means ± one SE.

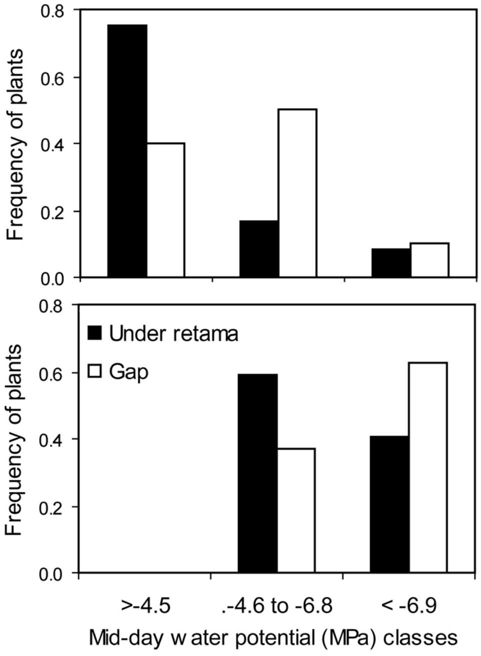

Figure 5. Frequency of *Q. ilex* seedlings according to midday leaf water potential classes under the *Retama* canopy and in gaps in 2006 (above) and in 2007 (below). The number of sampled plants under the *Retama* canopy and in gaps in 2006 was 12 and 10, respectively, whereas 24 plants per position were sampled in 2007.

2007 (year × position interaction, $\chi^2 = 6.20$, $P = 0.045$; Fig. 5). Neither herb competition ($\chi^2 = 0.13$, $P = 0.94$) nor plant size ($\chi^2 = 5.00$, $P = 0.08$) had any effect on Ψ.

Discussion

Retama sphaerocapa facilitated *Q. ilex* seedlings, which is consistent with greater recruitment of this oak under the canopy of the *Retama* than in the gaps observed in other *Retama* shrublands (Tovar 2009). This study provides further evidence for the role of facilitation in the secondary succession of Mediterranean ecosystems (Maestre *et al*. 2001; Gómez-Aparicio *et al.* 2004). However, the mechanism underlying facilitation in this system is complex since it differed between years and depended on seedling size.

Temporal variation in the relative importance of direct and indirect facilitation

In both years, *Retama* facilitated oak seedling survival, whereas herbs reduced it, demonstrating that herbs competed with oak seedlings. However, the effects of *Retama* and herbaceous vegetation on seedling survival differed between years. Herb competition started earlier in the growing season in the average rainfall year than in the humid year as indicated by early higher spring mortality that occurred in the former relative to the latter year. This can be explained by the dryer spring of the average rainfall year in comparison to the humid year. Our results suggest that there is a link between the prevailing facilitation mechanism and climate conditions. Facilitation was predominantly indirect in the average rainfall year, when spring was drier than in the humid year. By contrast, facilitation was direct in the humid year, when summer was drier than in the average rainfall year. This assertion is supported by the following results. First, herbs increased oak mortality in gaps but not under the *Retama* canopy in the average rainfall year (Fig. 2). The lower herb mass found under *Retama* as compared to the gaps, specially in the average rainfall year, suggests that the nurse shrub reduced the competitive capacity of the herbs, and hence indirectly improved seedling survival, as previously reported in other studies (Li and Wilson 1998; Siemann and Rogers 2003; Kunstler *et al.* 2006). Additionally, species composition of the herbaceous community differed between years. In the average rainfall year, graminoids predominated over non-graminoids under the nurse shrub, whereas both herb guilds had similar abundance in gaps. By contrast, in the humid year, graminoid and non-graminoid cover were similar both under the *Retama* and in the gaps. Differences in species composition may affect the competitive capacity of the community (Gordon *et al.* 1989), and graminoids tend to prevail under drought conditions (Goldberg *et al*. 2001). Secondly, seedling mortality in the absence of herbs did not differ between gaps and under the nurse shrub in the average rainfall year, but it did during the humid year. This demonstrates lack of direct facilitation in the former case, but the existence of direct facilitation in the latter case. In the average rainfall year, resource competition between the nurse shrub and oak seedlings may have overwhelmed the direct positive effects of *Retama* canopy on oak seedlings (Ludwig *et al.* 2004; Maestre and Cortina 2004). Third, the reduction in oak seedling survival caused by herbs was similar under the shrub and in the gaps in the humid year, suggesting a lack of indirect facilitation. Consistently, relative differences in herb mass or in the composition of the herbaceous community were smaller or non-existent between positions in the humid year respect to the average rainfall year.

Thus, in response to our first question, indirect facilitation may also be an important mechanism for oak regeneration in Mediterranean shrublands that are mostly known for direct facilitation (Pugnaire *et al*. 1996b; Gómez-Aparicio *et al*. 2004; Pugnaire *et al*. 2004). In addition, the mechanism underlying facilitation may vary with climatic conditions. Indirect facilitation can be important in years of dry springs where competition for water between nurse shrubs and herbs is high, resulting in benefits for oak seedling establishment, whereas direct facilitation mediated by microclimate amelioration seems to increase with summer aridity. Therefore, not only summer drought but also spring climatic conditions may determine facilitation interactions. This hypothesis should be experimentally tested in the future.

Underlying mechanisms of direct and indirect facilitation

Variations in soil nutrient and water content do not explain the direct facilitation observed in our system. Soil fertility did not differ between positions, probably because our nurse shrubs were too young to have induced changes in soil properties (Pugnaire *et al*. 1996b). Soil water content was lower or similar under the *Retama* canopy in comparison with the gaps, as documented in other dry environments (Tielbörger and Kadmon 2000; Valladares and Pearcy 2002). Irradiance reduction seemed to be the main driving force for oak seedling direct facilitation. The lower irradiation measured under the shrub explains the lower leaf temperature found in this microsite, which probably reduced oak transpiration under *Retama* (Smith and Geller 1980). Consequently, oak seedlings were less water-stressed and photo-inhibited under the nurse shrub than in the gaps, which could explain their higher survival rate under the shrubs (Baquedano and Castillo 2006; Hikosaka *et al* 2004). The higher Ψ and F_v / F_m values found under *Retama* relative to gaps support this argument. Therefore, the physiological results of this study provide a mechanistic explanation for the role that moderate shade plays in direct facilitation in dry environments such as Mediterranean ecosystems (Maestre *et al*. 2001; Gómez-Aparicio *et al*. 2005b). Seedlings were less water stressed and photo-inhibited (Fig. 4 and Fig. 5) in the average rainfall year than in the humid year. The higher summer rainfall and soil water content in the former year can explain this difference.

Levine (1999) predicted that indirect facilitation is more probable when different pairs of competitors compete for different resources or have different mechanisms to acquire them. The three competitors of our particular system are likely to compete for the same resource, *i.e*. soil water. We suggest that nurse shrubs and herbs competed mainly for soil water because the soil water content under *Retama* shrubs in spring was lower than in gaps in both years. However, *Retama* only suppressed herb competitive capacity in the average rainfall year, probably because soil water content under the *Retama* canopy with herbs in spring was 20% lower than the soil water content in the spring of the humid year. The reduction in soil water content imposed by the *Retama* under its canopy did not hinder oak performance probably because it is more drought tolerant than herbs (Levitt 1980; Larcher 1995).

Many studies have shown that shading by adults is the main factor responsible for reducing the competition of herbs with tree seedlings (Li and Wilson 1998; Pagès *et al*. 2003; Siemann and Rogers 2003; Kunstler *et al.* 2006). In our study, shade *per se* is unlikely to be a major mechanism of herb suppression because shrub shade was similar in both years but shade could have exacerbated the water stress caused by low soil water content in the spring of the average rainfall year (Valladares and Pearcy 2002). In contrast to our results, some previous studies have found greater herb biomass under *Retama* shrubs than in gaps (Pugnaire *et al*. 1996b; Rodríguez-Echeverría and Pérez-Fernández 2003). This can be attributed to the extremely dry climate of some regions, where facilitative interactions can outweigh the competitive interactions, and to the fact that these studies were performed on grazed woodlands, where herbivores may reduce herb biomass to a greater extent in open spaces than under shrubs (Osem *et al.* 2007).

Herbs and oak also competed for water because herbs reduced water availability for oak seedlings in spring (Fig. 1) as observed in previous studies (Rey Benayas *et al*. 2005; van der Waal *et al*. 2009). This can be due to herb canopy interception of rainfall or by direct exploitation by roots. Competition for water has been shown to be a major interaction between herb species and tree seedlings in water-stressed systems (Knoop and Walker 1985; Ludwig *et al*. 2004; van der Waal *et al*. 2009), while competition for nutrients is more commonly involved in mesic systems (Pagès and Michalet 2003). Nevertheless, herbs and oaks might also compete for soil nutrients, and diminution of herb competition by nurse shrub might have increased nutrient availability to the oak seedlings (Pagès *et al*. 2003).

Response to facilitation depended on oak seedling size

The response to facilitation has been demonstrated to vary among species (Gómez-Aparicio *et al*. 2004; Liancourt *et al*. 2005; Padilla and Pugnaire 2009). Our study goes a step beyond and indicates that not all individuals within a species have the same response to facilitation. Thus, amelioration of abiotic and biotic conditions by the *Retama* benefited smaller seedlings but not larger seedlings since the *Retama* reduced mortality of smaller seedlings relative to gaps but did not for larger seedlings. Large seedlings frequently have lower mortality than small seedlings under higher stressful conditions (Cook 1980; Leishman and Westoby 1994; Villar-Salvador *et al*. 2008), such as those found in gaps. This can be explained on the basis that large seedlings probably contain more nutrient and storage compounds (Cook 1980; Leishman and Westoby 1994; Villar-Salvador *et al*. 2008) and have higher photosynthesis (Cuesta unpublished data) than smaller seedlings. Differences in seedling size within a population can be attributed to genetic differences among individuals as demonstrated for *Q. ilex* (Leiva and Fernández-Alés 1998), but also to emergence time or seed size (Jurado and Westoby 1992; Green and Juniper 2004; De Luis e*t al*. 2008). We suggest that if oak seedling size has a genetic basis and determines mortality probability, nurse shrubs may contribute to maintain genetic diversity of oak populations.

Conclusions

Both indirect and direct facilitation are important processes of oak regeneration in *Retama* shrublands. The pioneer shrub *R. sphaerocapa* facilitated *Q. ilex* seedlings both by suppressing herb competition (indirect facilitation) and/or by ameliorating microclimatic conditions under its canopy (direct facilitation). Prevalence of indirect and direct facilitation differed between years of distinct climatic conditions. Indirect facilitation seems to be important in years of dry springs, whereas direct facilitation may increase with summer aridity. Since large areas of the Iberian Peninsula have been planted with or colonised by pioneer shrubs, and seedling stage is the most critical phase in Mediterranean woodland regeneration, nurse shrubs could enhance shrubland conversion into forests and play an important role in both passive (*i.e*. secondary succession) and active restoration of Mediterranean woodlands (Padilla and Pugnaire 2006; Rey Benayas *et al*. 2008). Furthermore, future studies should assess the incidence of indirect interactions among plants in other Mediterranean shrublands, in which direct positive interactions are known to drive community structure.

Acknowledgments

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Enrique de la Montaña Andrés

Capítulo 5

Capítulo 5

Los rasgos de vida en la encina responden de forma diferente a ambientes experimentales previos

Este capítulo reproduce íntegramente el texto del siguiente manuscrito:

Rey Benayas, J. M., Cuesta, B., Villar-Salvador, P. and Jaúregui, P. 2008. Life-history traits in an evergreen Mediterranean oak respond differentially to previous experimental environments. Web Ecology 8: 74-83.

Resumen

Los seres vivos responden tanto al ambiente presente como al ambiente pasado, lo que puede tener importantes consecuencias en la dinámica de poblaciones. Sin embargo, hay pocas evidencias experimentales basadas en estudios de campo a largo plazo que analicen el efecto que el ambiente previo tiene en el desarrollo de los individuos. Testamos la hipótesis de que los árboles que se establecen bajo condiciones ambientales distintas responden de forma diferente a las mismas condiciones post-transplante. La especie estudiada fue la encina, *Quercus ilex* subsp. *rotundifolia*, una especie mediterránea de crecimiento lento. Analizamos el efecto del ambiente previo, de la competencia intraespecífica y de los compromisos entre rasgos de vida (supervivencia, crecimiento y reproducción). El establecimiento de los plantones durante los tres primeros años fue facilitado reduciendo el estrés abiótico mediante riego estival y sombra artificial en 12 parcelas experimentales, mientras que cuatro parcelas se dejaron como parcelas control. Posteriormente, estos tratamientos fueron interrumpidos durante 10 años. Los plantones bajo condiciones ambientales suavizadas sobrevivieron y crecieron más durante el establecimiento inicial. Durante el periodo post-manejo, los tratamientos previos 1) no tuvieron ningún efecto en la supervivencia, 2) disminuyeron el crecimiento aéreo, 3) redujeron el crecimiento radical como indicó la reflectividad de un Sistema Georadar, 4) incrementaron la producción de bellotas principalmente por un mayor volumen del dosel y 5) aumentaron el esfuerzo de producción de bellotas. Los árboles experimentaron una combinación de efectos relacionados con la aclimatación al estrés abiótico y de efectos relacionados con la competencia intraespecífica. De acuerdo con nuestra hipótesis, el desarrollo de los árboles dependió de las condiciones ambientales previas, y la respuesta fue diferente para los distintos rasgos de vida. El manejo previo es recomendado porque incrementa la cobertura vegetal, reduce el tiempo necesario para alcanzar la madurez sexual e incrementa la producción de bellotas. Parcelas como las ensayadas en este trabajo pueden actuar como fuentes de propágulos en paisajes agrícolas deforestados, impulsando el establecimiento natural de nuevas plantas.

Palabras clave: asignación de recursos, competencia, compromiso, crecimiento, esfuerzo de producción de bellotas, *Quercus ilex.*

Life-history traits in an evergreen Mediterranean oak respond differentially to previous experimental environments

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Abstract

Living organisms respond both to current and previous environments, which can have important consequences on population dynamics. However, there is little experimental evidence based on long-term field studies of the effects of previous environments on the performance of individuals. We tested the hypothesis that trees that establish under different environmental conditions perform differently under similar post-establishment conditions. We used the slow-growing, evergreen Mediterranean oak *Quercus ilex* subsp. *rotundifolia* as target species. We analyzed the effects of previous environments, competition effects and tradeoffs among life-history traits (survival, growth, and reproduction). We enhanced seedling establishment for three years by reducing abiotic environmental harshness by means of summer irrigation and artificial shading in 12 experimental plots, while four plots remained as controls. Then these treatments were interrupted for ten years. Seedlings under ameliorated environmental conditions survived and grew faster during early establishment. During the post-management period, previous treatments 1) did not have any effect on survival, 2) experienced a slower above-ground growth, 3) decreased root biomass as indicated from reflectivity of Ground Penetration Radar, 4) increased acorn production mostly through a greater canopy volume and 5) increased acorn production effort. The trees exhibited a combination of effects related to acclimation for coping with abiotic stress and effects of intra-specific competition. In accordance with our hypothesis, tree performance overall depended on previous environmental conditions, and the response was different for different life-history traits. We recommend early management because it increased plot cover, shortened the time to attain sexual maturity and increased the amount of acorn production. Plots such as those assessed in this study may act as sources of propagules in deforested agricultural landscapes thus aiding natural establishment of new plants.

Key words: acorn production effort, competition, growth, *Quercus ilex*, resource allocation, trade-off.

Introduction

While genotype determines the potential performance of an individual, the multiple environmental conditions experienced during development and growth shape the resulting phenotype. Thus, performance of organisms can be influenced by both current and previous environments (Bazzaz 1996; Reekie *et al*. 1997; Metcalfe and Monaghan 2001; Weinig and Delph 2001; Relyea 2002; Grether 2005). However, whereas many basic and applied studies have addressed the effects of the manipulation of the current environment on the performance of organisms (Valladares *et al*. 2002; Castro *et al*. 2004; Rey Benayas *et al*. 2005), there is little experimental evidence of the effects of previous environments on the future performance. This type of experiments is scarce in the scientific literature.

Life-history theory predicts the existence of tradeoffs among traits, particularly survival, growth and reproduction, that can have important long-term consequences on population dynamics (Sadras 1996; Silvertown and Dodd 1999; Obeso 2002; Gilbert *et al*. 2006; Yoshida 2006). Tradeoffs are usually more apparent when resources are limited (Obeso 2002), as occurs in Mediterranean ecosystems. To determine the mechanistic bases of the effects of previous environments, we analyzed the consistency of the response for different life-history traits and their tradeoffs.

Most studies with woody species have focused on tradeoffs between growth and reproduction at the shoot level (Newell 1991), and less frequently at the entire plant or stand levels (Despland and Houle 1997). Further, hardly any investigations of reproduction costs have studied how these vary with plant size (Bañuelos and Obeso 2004; Sakai *et al*. 2006). Resources for plant growth can be allocated to both belowground and aboveground tissues. Because root biomass of adults is difficult to estimate, most studies of tree species addressing belowground growth use young individuals either planted in pots or grown in the field (Reich *et al*. 1998; Canham et *al*. 1999).

We aim to analyse the responses of several life-history traits of the slow-growing, evergreen Mediterranean oak *Quercus ilex* subsp. *rotundifolia* (*Fagaceae*) to previous environments in experimental plots. Moreover, we analyse the existence of tradeoffs between survival, growth, reproduction and biomass allocation. Environmental harshness was mitigated for three years to favour seedling establishment. Reduction of light and water stress increased seedling survival and growth rate (Rey Benayas 1998). After three years management ceased and saplings grew for ten years under natural, more severe environmental conditions. Previous research highlighted that trees that established under less stressful environmental conditions attained sexual maturity earlier than trees in control plots (Rey Benayas and Camacho-Cruz 2004). Due to differential survival and growth during the first three years, these plots represent a range of intra-specific competition intensities.

We tested the hypothesis that trees that established under different environmental conditions respond differently to homogenous post-establishment environmental conditions. There are two possible types of response to the positive effects provided by previous management: inertia, *i.e.* plants retained the ability to respond favourably to natural environmental harshness, or reaction, *i.e.* plants were less capable of facing later environmental harshness. We expected that the type of response would vary among life-history traits with tradeoffs between root *versus* aboveground growth, survival *versus* growth, and growth *versus* reproduction. Reproductive individuals, which were more abundant in previously managed plots (Rey Benayas and Camacho-Cruz 2004), were expected to grow more slowly. We also expected that the conflict for the allocation of resources to growth and reproduction would be more intense in those plots that have allocated proportionally more resources to roots than to shoots because of the cost of root maintenance.

We considered two levels of analyses, the individual and the plot (aggregates of interacting individuals). Because observational approaches often cannot demonstrate causal relationships as they do not control for environmental effects (Reznick 1985), experimental approaches are essential to advance our understanding of how the interplay between previous and current environmental conditions influences tradeoffs among life-history traits. This research has important applications for the restoration of Mediterranean woodlands because *Q. ilex* is a widespread structural species that comprises most of the community biomass where it thrives. Thus, we expect to obtain useful insights for restoration of other woodlands in the world.

Material and methods

Field experiment

The study site was located in central Spain (40° 3´N, 4° 24´W, 450 m a.s.l.) and has a typical Mediterranean continental climate. Total annual precipitation is 480 mm and mean annual temperature is 15 °C. We conducted the experiment on a 1 ha plot on previous cropland where a plantation of *Q. ilex* seedlings was established in 1993. During the first three years, seedlings were subjected to one of four treatments arising from the combination of summer irrigation (presence or absence) and artificial shading (presence or absence), with four replicates per combination. The 16 treatment plots were 10 × 10 m. They were planted with 50 one-year-old seedlings separated 2 m from each other with a regular distribution, 800 seedlings in total. Seedlings were previously cultivated in a nursery from acorns that were collected in a nearby locality to minimize genetic variation (Rey Benayas 1998). High plantation density eventually resulted in intense competition within plots.

Measurements

Treatments were halted in 1996, after which trees experienced natural rainfall and light conditions. At that time sapling density was different among treatments (Fig. 1) and plants were pre-reproductive. The plots were revisited in 2002-2005 to evaluate further performance of the trees. No other woody plant species had established in the plots. We measured: 1) mortality, 2) the relative growth rate (RGR) in tree volume (height × crown projected area), which was calculated for the periods 1996-2004, 2002-2004 and 2004-2005, 3) canopy volume in a plot, *i.e.* the sum of the individual tree volumes, 4) plot ground reflectivity along a 2.2 m depth profile, a surrogate of plot root biomass, was measured using Ground Penetration Radar (GPR, $\lambda = 16$ cm) (Antúnez Aranha *et al*. 2002; Butnor *et al*. 2003). This estimates the volume of roots 2.5 cm in diameter or greater. The reflectivity in each plot was based on the signals of five 10 m tracks per plot and *ca.* one radar pulse per cm. To correct for ground signals other than roots, we measured the ground reflectivity in four adjacent herbaceous plots, and the average signal was subtracted from signal of the oak experimental plots. We note, though, that our reflectivity measures correspond to a fraction of the total roots. Ground reflectivity was not measured at the end of the management period, 5) shoot to root ratio in a plot was estimated as canopy volume/ground reflectivity, 6) acorn production: all acorns were collected from all reproductive individuals in 2004, dried at 80ºC for three days, and weighed. Acorn production effort of an individual was calculated as the ratio of acorn biomass and its canopy volume.

Data analysis

Our analytical unit was either the plot (stand level analysis) or the individual. At the plot level, we analyzed mortality rates, above- and belowground growth, and acorn production effort. We tested the tree response to previous environmental conditions and competition intensity by means of two-way ANCOVA (independent factors were irrigation and shading) and correlation analyses. Two measures were used as surrogates of competition intensity within a plot, tree density and canopy volume, which were marginally correlated (r = 0.44, *P* = 0.091). Tree density ranged between 18 and 48 individuals per plot and canopy volume ranged between 50.1 and 342.6 m^3 $plot^{-1}$ in 2005. We expected that tree density and canopy volume of the plot would influence some life-history traits, *e.g.* effects of competition intensity on survival and growth and of tree size on fecundity. Therefore, canopy volume was used as a covariate in the ANCOVA models. We did not use tree density as a covariate in the ANCOVA models to avoid redundant hypothesis testing and results. To know if the conflict for the allocation of resources to growth and reproduction would be more intense in plots with contrasting shoot to root ratios (control and irrigation treatments), we used an ANCOVA (the categorical factor was the treatment, the covariate was acorn production effort) to test for parallelism between this ratio and the dependent variable (RGR). We used Turkey's tests for comparisons among treatments.

At the individual level, we looked at the trade-offs between aboveground growth and acorn production effort. Since the individuals were aggregated in plots, we tested the significance of the effect variables (sexual maturity, growth prior to the reproduction event, and growth subsequent to the reproduction event) in models with plot as a covariate random factor by means of GLM. First, we compared RGR between reproductive trees and non-reproductive trees. Next, the relationship between these traits was analysed by means of linear regression to relate the acorn production effort in 2004 with prior growth (2002-2004) and subsequent growth (2004-2005). We used Spearman's rank correlation instead of linear regression for cost of prior growth on reproduction because the residuals were not normally distributed due to high dispersion at low RGR values. All statistical analyses were performed with STATISTICA 6.0 Package (StatSoft, Inc., Tulsa, OK, USA).

Results

Mortality and growth

Treatments reduced mortality while they were applied, though mortality counts in control plots were highly variable (Fig. 1). Mortality among treatments during the post-management period ranged from 0% in control plots to 4%

in shaded plots. These differences were not significant (Table 1) and were not related to competition intensity (Table 1; correlation between post-management mortality and density was r = 0.07, $P = 0.8$).

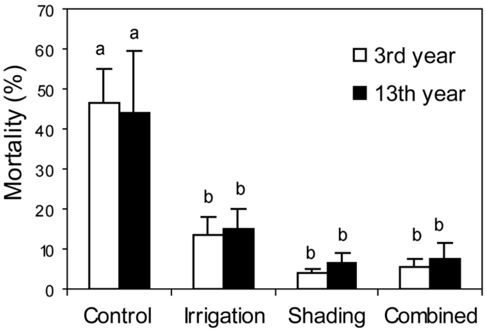

Figure 1. Shaded bars: cumulative mortality of planted *Quercus ilex* trees under different combinations of summer irrigation and artificial shading applied for three years and an additional 10 year post-treatment period ($F_{3,11} = 9.32$, $P = 0.0015$, $r^2 = 68.9\%$). Open bars: mortality at the end of the treatment period reported by Rey Benayas (1998). Different letters mean statistical differences within a year (3rd or 13th) at $P = 0.05$ according to a Turkey's test. Data are mean values ± SE. *Note:* mortality counts during the entire 13 year period are lower than mortality counts during the first three years in control plots because resprouting of apparently dead saplings outweighed additional mortality.

Previous management, particularly artificial shading and canopy volume caused a decrease in growth rate during the 1996-2004 post-management period (Table 1, Fig. 2). Relative growth rate was also negatively correlated with current plot tree density (r = -0.76, $P < 0.001$). At the end of the experiment, irrigated plots had greater canopy volumes, but only in non-shaded conditions (irrigation × shade interaction $F_{1,12} = 5.85$, $P = 0.032$). However, the differences in canopy volume among treatments were small ($F_{3,12} = 2.47$, $P = 0.11$). Post-management mortality and RGR were not correlated (r = -0.21, $P = 0.44$).

Ground reflectivity was higher in control plots than in previously managed plots (Table 1, Fig. 3). It was not related to canopy volume but it was negatively correlated with tree density (r = -0.65, $P = 0.006$).

Previous management increased the shoot to root ratio at the end of the experiment (Table 1, Fig. 3). Shoot to root ratio was positively related to plot density (r = 0.52, $P = 0.04$). During the entire post-management period and during

Table 1. Results of the ANCOVA models used to test for the effects of previous environmental conditions (summer irrigation and artificial shading applied for three years) and canopy volume on several performance attributes of *Quercus ilex* trees during the post-management period. Error df are 11.

Source	df	Mortality		Volume RGR		GPR reflectivity		Shoot to root ratio		Acorn production	
		F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
Summer Irrigation	1	1.14	0.31	4.79	0.051	2.43	0.15	0.70	0.42	1.6	0.23
Artificial Shading	1	0.18	0.68	80.59	0.000	7.37	0.02	8.14	0.016	2.93	0.11
Irrigation*Shade	1	0.98	0.34	8.52	0.014	3.53	0.09	4. 90	0.04	5.60	0.038
Canopy volume	1	1.20	0.29	4.74	0.052	0.06	0.81	263.0	<0.001	107.40	<0.001
Model (F, R^2 %)	4	0.54, 14%	0.71	23.17, 85.5%	<0.001	5.85, 49.7%	0.01	128.1, 97.1%	<0.001	34.84, 90.0%	<0.001

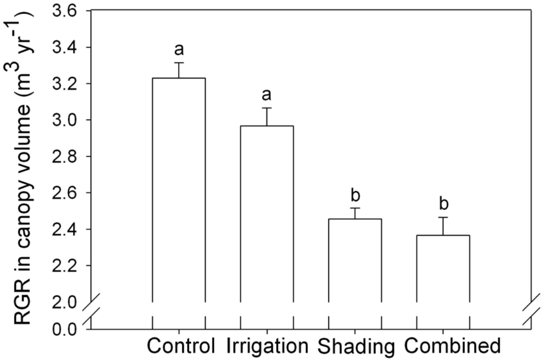

Figure 2. Relative growth rate in canopy volume of planted *Quercus ilex* trees during the post-treatment period after being previously treated by a combination of summer irrigation and artificial shading for three years. Different letters indicate statistical differences at $P = 0.05$ according to a Turkey's test. Data are mean values ± SE.

the 2004-2005 period, RGR was not correlated with the shoot to root ratio (r = -0.1, $P = 0.81$ and r = -0.43, $P = 0.096$, respectively).

Growth and reproduction

Acorn production in a plot depended on canopy volume and the irrigation and shading interaction (Table 1); more voluminous canopies produced more acorn biomass, and previous irrigation increased acorn production only in full-light plots. Acorn production effort and the shoot to root ratio were positively correlated (Fig. 4). Relative growth rate in the treatments with the most contrasting shoot to root ratios (*i.e.* control plots and irrigated plots) was not affected by the interaction between treatment and the acorn production effort ($P = 0.37$); this means that the slopes of RGR *vs* acorn production effort were similar in the contrasting plots.

GLM individual-based models including the plot as a random factor indicated significant effects of sexual maturity on growth ($P = 0.001$) and of

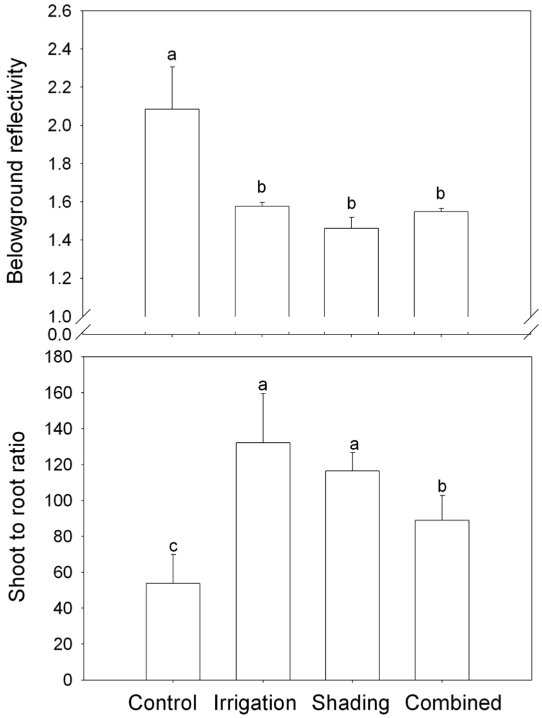

Figure 3. Ground reflectivity (above) and shoot-to-root ratio (below) in plots of planted *Quercus ilex* trees after 13 years having previously been treated by a combination of summer irrigation and artificial shading for the first three years. Different letters above the bars indicate statistical differences at $P = 0.05$ according to a Turkey's test. Data are mean values ± SE.

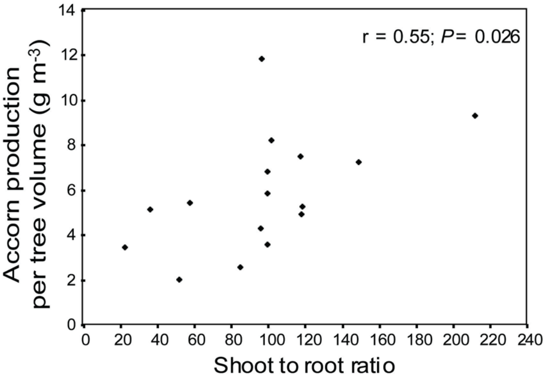

Figure 4. Relationship between acorn production per canopy volume unit and shoot-to-root ratio in plots of planted *Quercus ilex* trees after 13 years (n = 16).

previous growth on reproduction ($P < 0.001$), but not of reproduction on subsequent growth ($P = 0.37$). Reproductive trees in 2004 (n = 242) grew more slowly than non-reproductive trees (n = 399) (mean 2004-2005 RGR ± SD were 0.24 ± 0.22 m^3 yr^{-1} and 0.32 ± 0.27 m^3 yr^{-1}, respectively; t = - 4.24, $P < 0.001$). The individual acorn production effort was negatively correlated with the growth in the previous two years (Fig. 5). This figure highlights low acorn production effort for high previous growth, whereas there is a range of acorn production effort for low to medium previous growth. Similarly, growth after the reproduction event (2004-2005) was negatively correlated with acorn production effort during 2004 (r = -0.15, $P < 0.001$, n = 583).

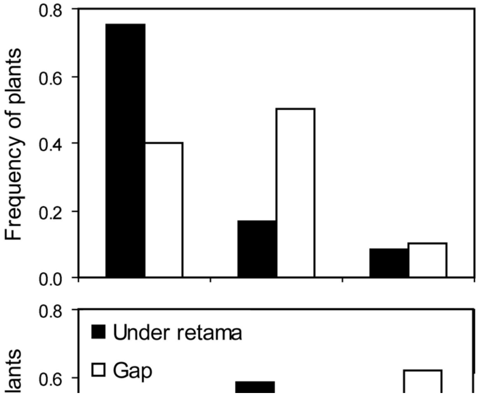

Figure 5. Relationship between current acorn production per tree volume and previous relative growth rate in canopy volume (2002-2004) in plots of planted *Quercus ilex* trees after 13 years (n = 635).

Discussion

Tree response to previous environments

Mitigation of stress due to high light and low water resulted in lower mortality and higher growth rate (Rey Benayas 1998). At that time, competition between saplings within a plot was negligible. In accordance to our hypothesis, trees that established under different environmental conditions overall responded differently to homogenous post-establishment environmental conditions, and the response was different for different life-history traits.

Previous environmental conditions did not affect subsequent mortality, an inertia response (Fig. 1). Many studies have demonstrated that the first year is critical for seedling establishment (Castro *et al.* 2004; Villar-Salvador *et al*. 2004; Verdú and Traveset 2005). Under stressful conditions, survival usually follows a type-III or Weibull model, whereas under less stressful conditions survival follows a linear-hazard or type-II exponential model (Rey Benayas *et al*. 2005). There was little mortality in the post-establishment years after stress mitigation ceased. Low post-establishment mortality may be due to the development of deep roots that allowed trees to avoid drought and to aerial growth that made trees less sensitive to herb competition.

Post-management aerial growth was higher in control plots; the saplings that grew faster during establishment slowed down their growth later, a reaction ('pay later') response. This response was clear for previously shaded plots, and marginally significant for previously irrigated plots, coinciding with low mortality but little above-ground growth in established seedlings during the treatment period (Rey Benayas 1998). This result suggests that growth is a flexible trait regulated at optimal rates rather than at maximal rates (Edenius *et al.* 1993; Arendt 1997). We attribute the reduction in growth to a combination of different processes.

First, plants grow more slowly as they become larger (van den Driessche 1992) because the proportion of photosynthetic tissues and the net assimilation rate declines with plant size (Walters *et al*. 1993). Secondly, there was higher intra-specific competition among trees in plots with previously ameliorated environmental conditions, as indicated by the negative relationships between RGR and canopy volume and density. Higher growth compensated for the high mortality due to abiotic stress in control plots; trees grew more at lower competition intensity than at higher competition intensity (Shabel and Peart 1994; Poorter *et al*. 2005). Third, after removing the effect of intra-specific competition, we still detected negative effects of previous management on growth, which may be a consequence of acclimation to cope with higher abiotic stress (photo-inhibition, water shortage) after management interruption (Hikosaka *et al*. 2004). Fourth, previous management accelerated growth and trees in control plots achieved sexual maturity later (Rey Benayas and Camacho-Cruz 2004). This experiment demonstrated that reproductive individuals grew more slowly than non-reproductive individuals. Fifth, there was a tradeoffs between aboveground growth and reproduction.

Tradeoffs between life-history traits

Tradeoffs for resource allocation also explain the observed effects of previous environments. Root mass, as estimated by ground reflectivity, was higher in control plots in spite of the lower density. Root mass and canopy volume were not correlated, pointing to little competition for resources between roots and shoots (Willaume and Pagès 2006). The differences among treatments in shoot to root ratio are explained only by root growth as little differences existed in canopy volume. The diversion of resources to root growth is a well-documented mechanism of plant adaptation to water stress that allows the use of resources more effectively (Chapin *et al*. 1993; Canham *et al*. 1996; Cornelissen *et al*. 1996; Sadras 1996). Allocation to roots also may be related to carbohydrate storage (Sadras 1996; Naidu and De Lucía 1997; Cherbuy et al. 2001; Drexhage and Colin 2001). However, a higher allocation of resources to roots can reduce RGR (Chapin 1990; Lambers and Poorter 1992; Antúnez *et al*. 2001). RGR and absolute growth in *Q. ilex* seedlings have been found to be positively correlated with the shoot to root ratio (Leiva and Fernández-Alés 1998; Villar-Salvador *et al*. 2004). RGR and shoot to root ratio were not correlated in this particular experiment, suggesting that the growth and maintenance of roots did not impair aerial growth. A possible explanation is a better water status in plants with higher amount of root biomass that compensates for the costs of root maintenance (Leiva and Fernández-Alés 1998). In contrast, shoot to root ratio explained a significant amount of the variation in acorn production effort (Fig. 4). We interpret this result as a consequence of the high maintenance cost related to a low shoot to root ratio (Mooney 1972).

We found that non-reproductive trees grew faster than reproductive trees and that acorn production and growth was traded off. This suggests that vegetative and reproductive functions compete for common resources. The cost of prior growth on subsequent reproduction at the individual level was mostly a consequence of slow growth in plants with high reproductive investment (Fig. 5). These results are consistent with those of Newell (1991), Houle (2001), Obeso (2002) and Bañuelos and Obeso (2004) with other woody species. However, other authors have reported an absence of reproduction costs (Fox 1995;

Despland and Houle 1997). Observational studies mostly report absence of tradeoffs or positive correlations between growth and reproduction, whereas manipulative studies highlight more easily the existing tradeoffs (Méndez pers. comm.).

Growth and survival during the post-management period were not related. Thus, growth did not have a density-dependent demographic cost yet. However, individuals grew more slowly in denser plots (Table 1, Fig. 2) or if they had a high acorn production effort (Fig. 5). This indicates that demographic costs (self-thinning) of early increased growth due to favoured establishment and possibly of reproduction may appear soon, as it has been demonstrated for other tree species (Hoffmann and Alliende 1984; Weiner 1990; Fox 1995; Sadras 1996; Silvertown and Dodd 1999).

Application to plantation management

Abandoned cropland and other deforested areas can be planted with native shrubs and trees to reduce soil erosion, increase biological diversity and create carbon sinks. However, the environmental conditions of these areas differ largely from those of the sites where natural regeneration of trees occurs (Rey Benayas *et al*. 2002), and the success of re-vegetation projects requires appropriate management. Since management is expensive, it is usually applied during a limited period after plantation takes place. Then, management is interrupted and the seedlings face a different environment than when they were first established.

Our results have important applications for tree species used for reforestation programs such as *Q. ilex*, an exemplary structural species in many forest communities of the central and western part of the Mediterranean basin. A benefit pursued by any restoration practitioner is to attain the highest re-vegetation cover in the shortest time. Consequently, we recommend early management because it increased plot cover (though this benefit was diluted with the age of plantation once management was halted), shortened the time to attain sexual maturity and increased the amount of acorn production. Plots such as those assessed in this study may act as sources of propagules in deforested agricultural landscapes thus aiding natural establishment of new plants (Chambers 2000; Robinson and Handel 2000; Rey Benayas *et al*. 2008).

Conclusions

Current performance of organisms may be at least partially a consequence of previous environments. The interruption of the amelioration of environmental harshness during a three year establishment period did not have any effect on tree survival ten years later, decreased RGR, a response due to both previous treatments and competition effects, levelled-off canopy volume across treatment plots as a consequence of the combined inertia in mortality and reaction in aboveground growth, increased the shoot to root ratio through a diminished root growth, and lastly, increased acorn production effort whereas acorn biomass was tightly linked to tree size. We detected a tradeoff between growth and reproduction but not between these traits and survival and between above- and belowground growths. Long-term experiments under field conditions, like the one presented here, will be very valuable as a mean of optimizing resource investment in active restoration of woodland ecosystems of the world (Hooper et al. 2002).

Acknowledgements

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Sigue tus sueños, aunque a veces parezcan pesadillas.
No te quedes con la duda.

Ana Guerrero Gil

Capítulo 6

Capítulo 6

Restauración de las propiedades químicas del suelo en un campo agrícola mediterráneo abandonado tras la sucesión secundaria y la reforestación

Este capítulo reproduce íntegramente el texto del siguiente manuscrito:

Cuesta, B., Rey Benayas, J. M., Gallardo, A., Villar-Salvador, P. and González-Espinosa, M. Restoration of soil chemical properties in abandoned Mediterranean cropland after secondary succession and woodland reforestation. En revisión en Restoration Ecology.

Resumen

Se analizó la respuesta de las propiedades químicas del suelo a la restauración pasiva o sucesión secundaria (herbazal) y a la restauración activa mediante la plantación de *Quercus ilex* (encinar restaurado) en un campo agrícola abandonado hace 13 años, y se compararon con las propiedades en un cultivo agrícola activo y un encinar maduro de referencia cercanos. El pH del suelo fue mayor en el cultivo activo y menor en el encinar maduro, que mostró una mayor concentración de C que los otros tres usos del suelo. La concentración de N total no difirió entre usos del suelo. La mayor concentración de P_2O_5 y K se encontró en el cultivo activo, y la menor en el encinar maduro, mientras que el encinar restaurado y el herbazal presentaron valores intermedios. La disponibilidad de N mineral fue mayor en el encinar restaurado que en el herbazal, mientras que no se observaron diferencias significativas en las tasas de mineralización ni en la disponibilidad de PO_4^{3-}-P. Se analizó también la importancia del manejo previo de la restauración (riego estival y sombra) y de las condiciones ambientales actuales en las propiedades químicas edáficas del encinar restaurado. Tanto el ambiente previo, principalmente el riego, como las condiciones ambientales actuales, principalmente la comunidad herbácea, afectaron a estas propiedades. Se confirma que la dinámica de la composición química del suelo en ecosistemas mediterráneos es lenta, lo que resulta en pequeñas variaciones de ésta en un cultivo agrícola restaurado hace 13 años. No obstante, la restauración activa de bosques nativos puede acelerar la recuperación de las propiedades edáficas en comparación con la sucesión secundaria.

Palabras clave: ambiente previo, ecosistema de referencia, fertilidad edáfica, herbazal, plantación de árboles.

Restoration of soil chemical properties in abandoned Mediterranean cropland after secondary succession and woodland reforestation

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Abstract

We analyzed the response of soil chemical properties to secondary succession (old field) and to active restoration with planted *Quercus ilex* (restored woodland) in a Mediterranean cropland abandoned 13 years ago, and compared them to the properties in nearby active cropland and mature *Q. ilex* woodland. Soil pH was highest in the active cropland and lowest in the mature woodland, which showed higher C concentration than the other land uses. Total N concentration did not differ among land uses. P_2O_5 and K concentration were highest in the active cropland and lowest in the mature woodland, whereas the restored woodland and the old field had intermediated values. The availability of mineral N was higher in the restored woodland than in the old field, with no differences in mineralization rates or available PO_4^{3-}-P. We also evaluated the relevance of previous restoration management (summer irrigation and shading) in addition to current environmental conditions on soil chemistry in the restored woodland. Both, previous management, particularly irrigation, and current environmental conditions, mostly herbaceous community, affected soil chemistry. We evidence the slow dynamics of soil chemistry in Mediterranean ecosystems, which results in little variation of soil properties in restored cropland after 13 years. However, active restoration of native woodlands can hasten the recovery of soil properties in comparison with secondary succession.

Key words: old fields, previous environments, soil fertility, target ecosystem, tree planting.

Introduction

Large extents of cropland have been abandoned in semiarid Mediterranean areas during the last decades due to a number of ecological and socio-economic factors (Bakker *et al*. 2005; Rey Benayas *et al.* 2007). These areas can be left undergo into secondary succession (passive restoration) or subjected to active restoration that mostly consists on tree and shrub planting and their management. Overall, passive restoration has been more successful and with a lower cost than active restoration (Rey Benayas *et al.* 2008a). Nevertheless, active restoration may be needed when land degradation is high and natural vegetation recovery is impeded or takes place slowly (Rey Benayas 2005).

After cropland abandonment, soil chemical properties may strongly differ from natural soil properties due to the previous agricultural practices such as fertilization, ploughing, and harvesting (Koerner *et al.* 1997; Compton and Boone 2000). Soil nutrients and carbon content change significantly over time in the abandoned croplands as secondary succession takes place. Generally, soil carbon increases with time after abandonment due to biomass accumulation, whereas available phosphorous decreases because of its immobilization in the living plant biomass (Du *et al*. 2007; Dölle and Schmidt 2009). The dynamics of mineral nitrogen is controversial; different studies have found either an increase, a decrease, or no change in the amount of nitrogen after abandonment (De Kovel *et al*. 2000; Hooker and Compton 2003; Dölle and Schmidt 2009).

In semiarid Mediterranean ecosystems, secondary succession is usually slow, because regeneration of woody plants is hindered by factors such us extreme climatic conditions, poor soil fertility, and competition from herbaceous vegetation (Rey Benayas 2005; Vallejo *et al.* 2006). Mediterranean abandoned croplands under passive restoration are initially colonized by herbaceous vegetation which persists for a long time before woody vegetation is established (Bonet and Pausas 2004). Thus, restoration of these systems usually requires active restoration techniques. Soil chemical properties are expected to be different under these contrasting scenarios of Mediterranean woodland restoration, as vegetation recovery differs between them. To our knowledge, no study has compared the effect of passive restoration (secondary succession) *vs.* active restoration on soil chemistry after agricultural abandonment in dry Mediterranean environments.

Several studies have shown that plant species differ in their capacity to modify soil properties (Gallardo and Merino 1993; Vinton and Burke 1995; Cornelissen *et al*. 1999; Jeddi *et al*. 2009). Plant functional traits such as growth form, biomass allocation, tissue chemistry, and lifespan can significantly affect organic matter decomposition and nutrient dynamics (Hooper and Vitousek 1998; Carrera *et al*. 2009). Plants with low growth rates such as Mediterranean evergreen species exhibit high concentration of secondary compounds and chemical defenses against herbivores such as phenol-protein complexes of low decomposability that make difficult its decomposition (Aerts and Chapin 2000; Satti *et al*. 2003). By contrast, plants with high growth rates, such as herbaceous vegetation, show high N concentration in green tissues and low content of secondary compounds which turn into high quality litter of rapid decomposition (Carrera *et al*. 2009).

Quercus ilex (holm oak) is one of the species commonly used for woodland restoration in the Mediterranean Basin. It is a slow-growing, sclerophyllous evergreen oak that is a major structural component of the natural woodlands in Western European and Northern African regions. Holm oak produces large amounts of litter which can potentially incorporate high quantities of organic matter and nutrients to the soil (Gallardo 2003; Moreno *et al.* 2007). In addition, the micro-climatic conditions generated under tree canopies in dry climates (lower soil temperature and higher soil moisture) enhance microbial activity and increase litter decomposition and organic matter mineralization (Muscolo *et al*. 2007; Sariyildiz 2008). Thus, it may be expected to find higher nutrient availability under the holm oak canopy in comparison with open spaces dominated by herbs (Moreno *et al*. 2007). However, foliage produced by sclerophyllous species such as holm oak, which has high tensile strength, is of low decomposability (Cortez 1998; Fioretto *et al.* 2007). Additionally, holm oak canopy reduces irradiation hindering the development of herbs, which produce higher decomposability litter (Enriquez *et al*. 1993; Cornwell *et al*. 2008). Since the presence of holm oak canopy may have both positive and negative effects on soil nutrient dynamics, it is necessary to analyze its effect on soil chemistry restoration.

In the present study, we analyze the response of soil chemical properties to secondary succession (hereafter old-fields) and to active restoration with *Q. ilex* (hereafter restored woodland) in a Mediterranean cropland abandoned 13 years ago. We compared the soil chemical properties in the old-field and in the restored woodland plots with the soil chemical properties in a continuously cultivated cropland (hereafter active cropland) and in mature *Q. ilex* woodland used as reference ecosystem (hereafter mature woodland). Our study is part of a long-term research aiming to investigate the effects of experimentally manipulated environments on ecosystem processes in restoration projects of Mediterranean woodland (Cayuela *et al*. 2008; Rey Benayas *et al.* 2008b). In this context, we also evaluated the relevance of previous management in addition to current environmental conditions on soil chemistry of the actively restored woodland. We hypothesized that 1) along the gradient from cultivated cropland to mature woodland, soil chemical composition of the old-field and of the restored woodland will be situated at intermediate positions, 2) nutrient concentration and availability will be higher in the restored woodland than in the old-field, and 3) current soil chemical properties of actively restored woodland reflect both a carry-over effect of previous management and a response to current environmental conditions. Our experimental approach will provide useful insights to forecast effects of active ecological restoration on soil properties in comparison to passive restoration of abandoned cropland, as well as an opportunity to test how responses to previous environments affect the current soil chemical properties.

Material and methods

Study site

The study site was located in an abandoned cropland in central Spain (40° 3'N, 4° 24'W, altitude 450 m). It has a typical Mediterranean continental climate, with a mean annual precipitation of 480 mm and a mean annual temperature of 15 ºC. Summer is hot and dry while winter is cold with frequent frosts. The soil is a *Luvisol* type derived from sandstone arkoses.

The restored woodland plots were an experimental plantation of *Quercus ilex* subsp. *ballota* established in 1993 in a 1 ha plot which had been cultivated for grain for four decades until the oak seedlings were planted (Rey Benayas 1998). During the first three years, seedlings were subjected to one of four treatments from the factorial combination of summer irrigation and artificial shading (control, irrigation, shading, and irrigation and shading), with four replicate plots per treatment. The 16 treatment plots were 10 m x 10 m in size and were planted with 50 one-year-old seedlings separated 2 m from each other with a regular distribution. Irrigation was applied by sprinklers at the peak of the dry season (60 mm in July and August; 120 mm in total per year) and added uniformly to the whole plot area. The shading treatment consisted of a 68% reduction in incident radiation by placing a black polyethylene net that stood 2 m above ground. The shading and irrigation treatments were interrupted in the winter of 1996. All plots have experienced natural rainfall and light conditions ever since then. The plots were protected from herbivores (sheep, rabbits and hares).

The old-field plots (four plots of 10 x 10 m) were located close to the restored woodland plots and have been subjected to secondary succession since plantation of restored woodland plots took place. Additionally, four plots of 10 m x 10 m were sampled in an adjacent active cropland and in the most nearby mature oak woodland. The active cropland is fertilized following a standard scheme in the area: application of a fertilizer with inorganic nitrogen, phosphorous and potassium (70:35:35, 400 kg ha^{-1}) once a year plus another annual application of just inorganic nitrogen (27% concentration, 150 kg ha^{-1}). The mature woodland is a remnant patch that was located *ca*. 30 km northwest to the old-field, restored woodland, and active cropland, was dominated by holm oaks, but also a few individuals of *Quercus coccifera* and *Cystus ladanifer* may be found. In the area, there are not patches of mature woodland closer to the rest of the studied scenarios since virtually all arable soils on sandstone arkoses have been transformed into cropland since long time ago. The climatic conditions among the four land use types considered were identical.

Vegetation and litter characterization

Different variables related to vegetation structure and composition and plant litter were measured both in the old-field and in the restored woodland plots. In May 2006, five 50 x 50 cm quadrats were set in each plot with a regular distribution; in these quadrats, herbaceous above-ground biomass and herbaceous and oak litter mass were taken and weighted after drying at 60 ºC for two days. All vascular plant species were identified and recorded in nine 50 cm x 50 cm quadrats set at each plot with a regular distribution. All species were classified into guilds according to their functional attributes as graminoids, legumes and forbs. We calculated the mean value of each measured variable (herb biomass, oak and herb litter mass, and total herbaceous, graminoid, legume and forb cover) per plot. In the restored woodland plots, oak canopy volume was calculated as the sum of the individual oak tree volumes, estimating each oak volume as height × crown projected area in December 2005. Crown projected area of each oak was estimated as the elliptical surface of the crown projected onto the ground.

Soil chemical properties

Soil samples were taken in March 2006 from the four studied land use types (active cropland, old-field, restored woodland and mature woodland). We systematically collected three 20-cm deep soil samples per plot at one of the plot diagonals; the location of the three sampling points divided the diagonal in four segments of similar length. These samples were combined into one single composite sample per plot (28 composite samples in total). Fresh soil composite samples were sieved to separate plant material and fragments > 2 mm in size. In each composite sample, soil pH, carbon (C), total nitrogen (N), phosphorous (P_2O_5), and potassium (K) were measured. Soil pH was determined in a 1:2.5 mass:volume soil and water suspension. C was analyzed using $K_2Cr_2O_7$ in a H_2SO_4 environment. Total N was determined by Kjeldahl analysis with $SeSO_4$-K_2SO_4 as catalyst. For P_2O_5 we used the method reported by Burriel and Hernando (1950). K was analyzed according to MAPA (1986) using an Optic PLASMA ICP (Perkin- Elmer, model 4300 DV).

Additionally, ammonium (NH^{4+}-N), nitrate (NO^{3-}-N), total mineral N (NH^{4+}-N + NO^{3-}-N) concentrations, and the potential rates of ammonification, nitrification, and mineralization were measured only in the old-field and restored woodland plots. Potential net mineralization, nitrification, and ammonification rates were determined by incubating 5 g of dry soil of each of the composite sample with 15 g of pure sand and 6 ml of water for 14 days in the dark at 30 ºC. Mineral N was extracted with 100 ml of KCl 2 N, shaken for 1 h and the suspension filtered through 0.45 mm millipore filters. NH^{4+}-N and NO^{3-}-N in the extract were measured by colorimetry (indophenol blue method), using a microplate reader (Sims *et al*. 1995). Potential net mineralization rate was calculated as the difference between the NH^{4+}-N+NO^{3-}-N concentration before and after the incubation period. Potential net nitrification was the difference for NO^{3-}-N concentration over the same period. Potential ammonification rate was the difference between potential net mineralization rate and potential nitrification rate.

In May 2005, the availability of ammonium (NH^{4+}-N), nitrate (NO^{3-}-N), total N (NH^{4+}-N + NO^{3-}-N) and phosphate (PO_4^{3-}-P) in soils were assessed in the old-field and restored woodland plots using anionic and cationic exchange membranes (types I-100 and I-200, Electropure Excellion, Laguna Hills, California). Resin membranes were previously conditioned in the lab by immersing them in demineralised water at 82-90 ºC for 48 h. After conditioning, 2.5 x 2.5 cm resin membranes were glued on a plastic holder to facilitate insertion into the soil. A plastic rod joined to the plastic holder helped to locate the resin membranes in the field. This design kept the membrane ionic exchange capacity unaltered (Cain *et al*. 1999). Exchange resin membranes were introduced in the soil at ca. 10 cm-depth during 20 days. After being removed, the membranes were taken individually to the lab and dried at ambient temperature. The attached soil was removed, the plastic rod was cut and an extraction was performed with 50 ml of 2 MKCl by orbital spinning for 1 h at 200 rpm in 125 ml flasks. These extracts were used to calculate the quantity of NH^{4+}-N, NO^{3-}-N, and total N-mineral (NH^{4+}-N + NO^{3-}-N) by the indophenol blue method (Sims *et al*. 1995) and PO_4^{3-}-P by the molybdenum blue method (Allen *et al*. 1986), with a microplate reader. We used two anion and two cation exchange resin membranes that were placed at the two external sampling points of each plot diagonal explained above and the mean value per plot was calculated.

Effects of previous and current environmental conditions

The management treatments (summer irrigation and artificial shading) applied to the reforested woodland plots between 1993 and 1996 were considered as surrogates of the environmental conditions during the first three years (*i.e*. conditions during early establishment of the introduced *Q. ilex* seedlings).

We measured different variables to describe the effect of current environmental conditions in each of the 16 restored woodland plots. In addition to oak volume, herbaceous aboveground biomass and litter mass, we estimated the canopy openness and soil moisture. Canopy openness was assessed by means of hemispherical (or fish-eye) digital photographs taken just before sunrise with a Nikon Coolpix 4500 camera with a Nikon Fisheye Converter FC-E8 0.21x and analysed with WinPhot 5.00 software (Hans ter Steege, Utrecht University, 1996). We took photographs at nine sampling points per plot set with a regular distribution and the mean value per plot was calculated. Soil moisture in the first 10 cm was measured at five sampling points, regularly placed, per plot with a time domain reflectometer (TDR, Topp *et al*. 1980) on three dates (13 May, 23 May, and 7 June 2006), and the mean value per plot was calculated. To account for the effect of current environmental conditions, we used oak canopy volume, herbaceous mass, and composition of the herbaceous communities as explanatory variables. Across restored woodland plots, oak canopy volume was negatively correlated with canopy openness (r = -0.74; *P* = 0.001; n = 16) and soil water content (r = -0.52; *P* = 0.041; n =16), and positively correlated with oak litter (r = 0.73; *P* = 0.001; n = 16). Since these four variables were highly correlated, we just used canopy volume in the statistical analyses. In order to reduce the dimensionality of the species composition data set into one single variable, a non-metric multidimensional scaling (NMDS) was performed and the values for the first axis were selected as values of species composition.

Data analysis

Differences in soil pH and C, N, P_2O_5, and K concentration among active cropland, old-field, restored woodland and mature woodland were tested by one-way ANOVA. Differences in mineral N (NH^{4+}-N, NO^{3-}-N, and total mineral N) concentration and availability, PO_4^{3-}-P availability, ammonification, nitrification and mineralization rates, herb biomass and litter mass, and total, graminoid, legume and forb cover between old-field and restored woodland plots were analysed with Student´s t tests. For comparison among land-use types, we only used a subset of restored woodland plots, *i.e*. the reforested control plots where treatments for aiding seedling establishment were not applied.

In the restored woodland plots, differences in soil chemical properties, vegetation cover and litter mass among the four previous management treatments (control, irrigation, shading, and irrigation and shading) were analysed by means of two-way ANOVA, were irrigation and shading were the factors analysed. Finally, the variance of the soil properties was partitioned into different components by means of redundancy analysis (RDA) (Borcard *et al*. 1992).

We differentiated: direct effects of previous management, direct effects of current environmental conditions (oak canopy and herbaceous community), indirect effect of previous management through their effect on current environmental conditions (oak canopy and herbaceous community), and indirect effect of oak canopy through its effect on herbaceous community. The partition of the variance analysis allows estimating the effect of each single variable or the effects of a group of variable; thus, the effect of the herbaceous community refers to both herb community composition and biomass. Indirect effect has no degree of freedom and, therefore, they can not be tested for significance.

Data were checked for normality and homogeneity of variance, and were transformed when necessary to correct deviations from these assumptions. Differences between the levels of significant factors were determined using *post-hoc* Tukey´s tests. All statistical analyses were performed with Statistica 6.0. Package (StatSoft, Inc., Tulsa, OK, USA) and R 2.8 (R Development Core Team 2008).

Results

Effect of land use type on soil chemical composition

Soil chemical properties differed among the four land use types. Soil pH was significantly highest in the active cropland (6.30 ± 0.21) than in the mature woodland (5.52 ± 0.06), whereas old-field (5.70 ± 0.16) and restored woodland (5.71 ± 0.22) had intermediate pH values ($P = 0.045$). Mature woodland showed greater C concentration than the other three land use types, which did not differ among them (Fig. 1a). Total N concentration did not differ among land use types (Fig. 1b). The C:N ratio increased from 9.35 ± 0.41 in active crop-land to 16.38 ± 0.91 in mature woodland. Concentration of P_2O_5 (Fig. 1c) and K (Fig. 1d) was highest in the active cropland and lowest in the mature woodland, whereas old-field and restored woodland had intermediate values.

Old field versus restored woodland

Oak canopy was relatively closed in the restored woodland (56.1%), whereas in the old-field any oak was established (Table 1). Thus, whereas in the restored woodland there was a relatively high quantity of oak litter, there is not in the old-field. Herbaceous cover ($P = 0.03$) and biomass ($P = 0.02$) were higher in the old-field than in the restored woodland, which turned into higher herbaceous litter in the former than in the latter ($P = 0.04$, Table 1). Leguminous ($P = 0.113$) and graminoid ($P = 0.661$) cover were similar in both restoration types but forb cover was higher in the old-field ($P = 0.048$, Table 1).

The concentration of soil NH^{4+}-N was higher in restored woodland than in the old-field, whereas no differences were found for the concentrations of NO^{3-}-N or total mineral N (Fig. 2a). Availability of NH^{4+}-N, NO^{3-}-N and total mineral N were higher in the restored woodland than in the old-field (Fig. 2b). In both restoration scenarios, the availability of NH^{4+}-N was higher than that of NO^{3-}-N. Soil ammonification, nitrification, and mineralization rates did not differ between the old-field and the restored woodland (Fig. 2c). No differences between the old-field and the woodland were found for available PO_4^{3-}-P (6.39 ± 1.28 and 6.06 ± 0.41 μg dm-^{2} day, respectively; $P = 0.91$).

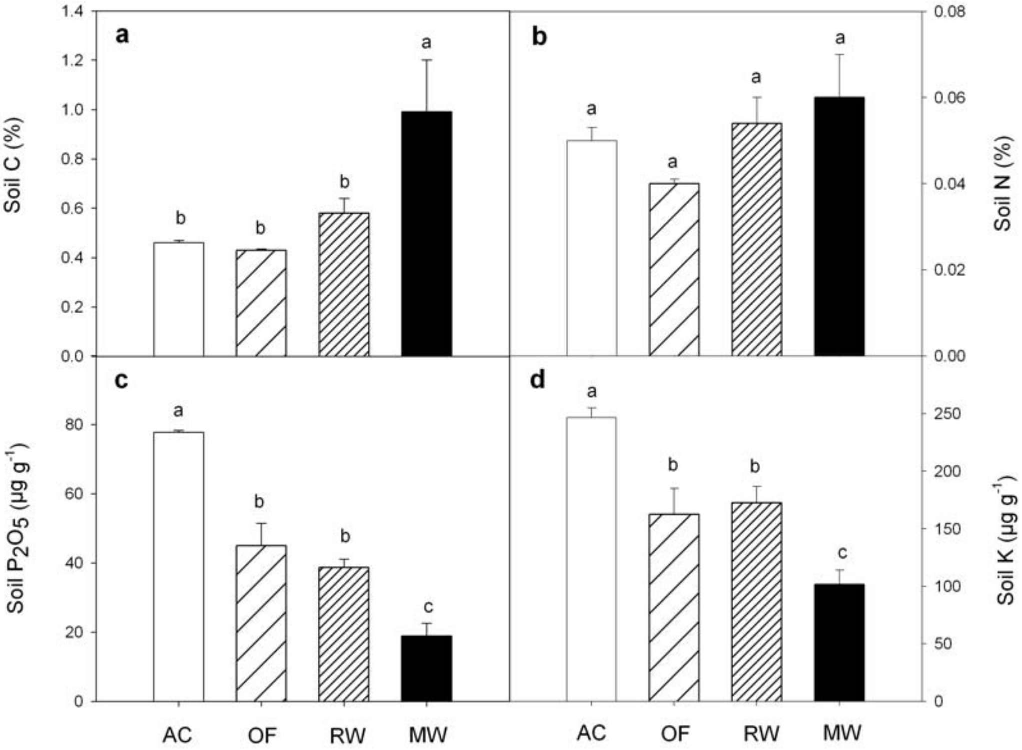

Figure 1. Soil C (**a**), total N (**b**), P2O5 (**c**), and K (**d**) measured in the active cropland (AC), old-field (OF), restored woodland (RW), and mature woodland (MW). Data are means ± 1 SE. Different letters mean statistical differences at $P < 0.05$.

Table 1. Vegetation cover and litter mass measured in the old field and in the restored woodland plots. Data are means ± 1 SE. Different letters mean statistical differences at $P < 0.05$. * These variables have not been statistically tested since the sample data are always 0 in the old field plots.

	Old field	Restored woodland
Oak volume (m^{3} ha^{-1}) *	0	103.1 ± 41.1
Oak litter mass (g m^{-2}) *	0	66.7 ± 24.0
Herbaceous biomass (g m^{-2})	143. 9 ± 12.35^{a}	82. 8 ± 15.6^{b}
Herbaceous litter (g m^{-2})	126.2 ± 20.67^{a}	78.1 ± 12.59^{b}
Herbaceous cover (%)	62.15 ± 4.59^{a}	58.2 ± 4.21^{b}
Legume cover (%)	3.69 ± 1.14	1.25 ± 0.77
Graminoid cover (%)	24.59 ± 4.82	24.3 ± 3.64
Forb cover (%)	33.86 ± 3.86^{a}	31.4 ± 2.74^{b}

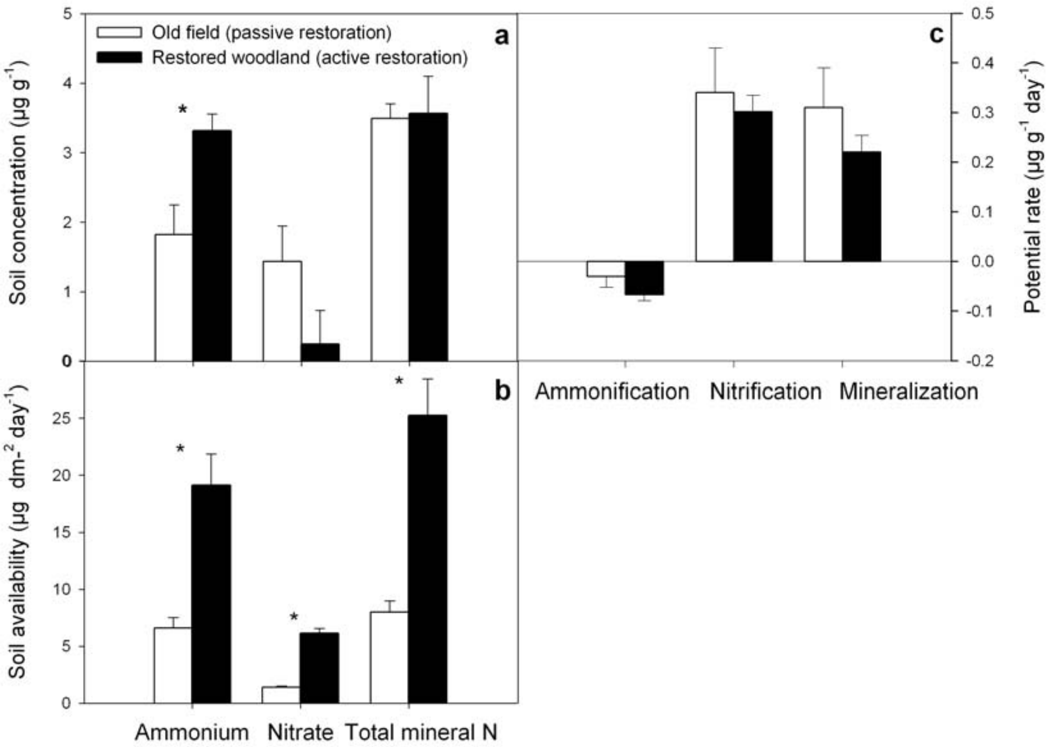

Figure 2. Soil ammonium, nitrate and total mineral N concentrations (**a**), soil ammonium, nitrate and total mineral N availability (**b**) and soil potential ammonification, nitrification and mineralization rates (**c**) measured in the old field and restored woodland plots. Data are means ± 1SE. * mean statistical differences at $P < 0.05$.

Effects of previous and current environmental conditions on soil chemical properties in restored woodland plots

Oak volume did not differ among the restored woodland plots subjected to different previous management treatments (Table 2). However, oak litter in the control plots was lower than in both irrigated and shaded plots. Herbaceous biomass and cover was lower in shaded plots, but there were no differences in herbaceous litter among the plots subjected to different previous managements (Table 2). Irrigation increased legume cover and shading reduced graminoid cover, but no treatment affected forb cover (Table 2).

Neither soil C nor total N differed among the restored woodland plots subjected to different previous management treatments. Soil K was highest in irrigated plots (either shaded or not) and lowest in shaded plots, whereas control plots had intermediate values (Table 2). Irrigated plots showed the highest P_2O_5 concentration, shaded and control plots the lowest, while the plots that had been both irrigated and shaded had intermediate values. Concentrations of NH^{4+}-N, NO^{3-}-N and total mineral N did not differ among previous treatment plots. By contrast, availability of NH^{4+}-N, NO^{3-}-N, and total mineral N differed among treatments (Table 2). Irrigated plots and shaded plots had higher NH^{4+}-N availability than the plots with both irrigation and shading, whereas

Table 2. Vegetation cover, litter and soil variables measured in the restored woodland plots under different treatments of previous management. Data are means ± 1SE. Different letters mean statistical differences at $P < 0.05$.

	Control	Irrigation	Shading	Irrigation and shading
Vegetation cover and litter mass				
Oak volume (m^{3} ha^{-1})	103.1 ± 41.1	209.8 ± 90.7	171.1 ± 42.1	137.8 ± 42.6
Oak litter mass (g m^{-2})	66.7 ± 24.0^{b}	127.5 ± 27.1^{a}	135.1 ± 7.74^{a}	83.1 ± 30.2^{ab}
Herbaceous biomass (g m^{-2})	82. 8 ± 15.6^{a}	54.9 ± 12.2^{ab}	35.1 ± 5.6^{b}	62.7 ± 18.4^{ab}
Herbaceous litter biomass (g m^{-2})	78.1 ± 12.59	68.4 ± 11.8	74.5 ± 5.88	65.5 ± 11.1
Herbaceous cover (%)	58.2 ± 4.21^{a}	45.1± 4.70^{ab}	32.0 ± 3.63^{b}	51.0 ± 10.6^{ab}
Legume cover (%)	1.25 ± 0.77^{bc}	4.19 ± 1.08^{ab}	0.75 ± 0.16^{c}	4.71 ± 1.71^{a}
Graminoid cover (%)	24.3 ± 3.64^{a}	15.9 ± 1.19^{ab}	11.5 ± 2.04^{b}	14.1 ± 2.83^{ab}
Forb cover (%)	31.4 ± 2.74	25.0 ± 4.04	19.8 ± 2.94	24.8 ± 2.30
Soil chemical properties				
pH	5.7 ± 0.22^{b}	6.5 ± 0.19^{a}	5.5 ± 0.05^{b}	6.5 ± 0.14^{a}
C concentration (%)	0.58 ± 0.06	0.53 ± 0.08	0.44 ± 0.06	0.55 ± 0.06
Total N concentration (%)	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.005	0.06 ± 0.01
K concentration (µg g^{-1})	172 ± 14.4^{ab}	257 ± 38.4^{a}	107 ± 6.29^{b}	230 ± 54.5^{a}
P_2O_5 concentration (µg g^{-1})	39 ± 2.39^{b}	90 ± 20.0^{a}	35 ± 3.54^{b}	63 ± 14.2^{ab}
NH_4^+-N concentration (µg g^{-1})	3.31 ± 0.24	1.88 ± 0.66	2.40 ± 0.43	2.83 ± 0.41
NO_3^--N concentration (µg g^{-1})	0.25 ± 0.49	0.47 ± 0.80	0.11 ± 0.87	0.78 ± 1.24
Total mineral N (µg g^{-1})	3.56 ± 0.54	2.35 ± 1.43	2.51 ± 1.17	3.61 ± 1.49
NH_4^+-N availability (µg dm^{-2} day^{-1})	19.1 ± 2.73^{ab}	24.3 ± 4.86^{a}	21.7 ± 1.02^{a}	10.7 ± 3.25^{b}
NO_3^--N availability (µg dm^{-2} day^{-1})	6.1 ± 0.45^{a}	6.1 ± 0.84^{a}	4.9 ± 1.45^{a}	1.9 ± 0.09^{b}
Total mineral N availability (µg dm^{-2} day^{-1})	25.2 ± 3.17^{a}	30.4 ± 5.41^{a}	26.6 ± 2.23^{a}	12.5 ± 3.31^{b}
PO_4^{3-} -N availability (µg dm^{-2} day^{-1})	6.06 ± 0.42	6.02 ± 0.77	7.50 ± 1.68	4.31 ± 0.34
Nitrification rate (µg g^{-1} day^{-1})	0.30 ± 0.03	0.21 ± 0.10	0.23 ± 0.09	0.21 ± 0.08
Mineralization rate (µg g^{-1} day^{-1})	0.22 ± 0.03	0.15 ± 0.09	0.23 ± 0.07	0.15 ± 0.08
Ammonification rate (µg g^{-1} day^{-1})	-0.08 ± 0.005	-0.05 ± 0.01	-0.01 ± 0.06	-0.06 ± 0.01

the control plots had intermediate values. NO^{3-}-N and total mineral N availability were lower in plots with both irrigation and shading than in the rest of the treatment plots, which did not differ among them. The four previous establishment treatments had no effect on PO_4^{3-}-P availability, as well as on potential rates of ammonification, nitrification, and mineralization (Table 2).

Direct effects of previous treatments at seedling establishment (*P* = 0.03, D1 in Fig. 3) and herbaceous community (*P* = 0.05, D3) explained most of the variation of soil chemical properties in the restored woodland plots (15% and 20% of variance accounted for, respectively), whereas oak canopy only explained 2% of the variance (*P* = 0.345, D2). Similarly, the indirect effects of previous establishment treatments through herbaceous community (I2) explained 16% of the variance, whereas indirect effects through oak canopy (I1) only explained 1.9 % of the variance. Oak canopy through it effect on herbaceous community (I3) indirectly explained 1.1 % of the variance.

Discussion

Land use and soil chemical properties

Our results showed that soil chemical properties differed among soils under different land use types. We also found that soil changes induced by agricultural practices may persist or have a signature long time after crop abandonment (Koerner *et al*. 1997; Compton and Boone 2000).

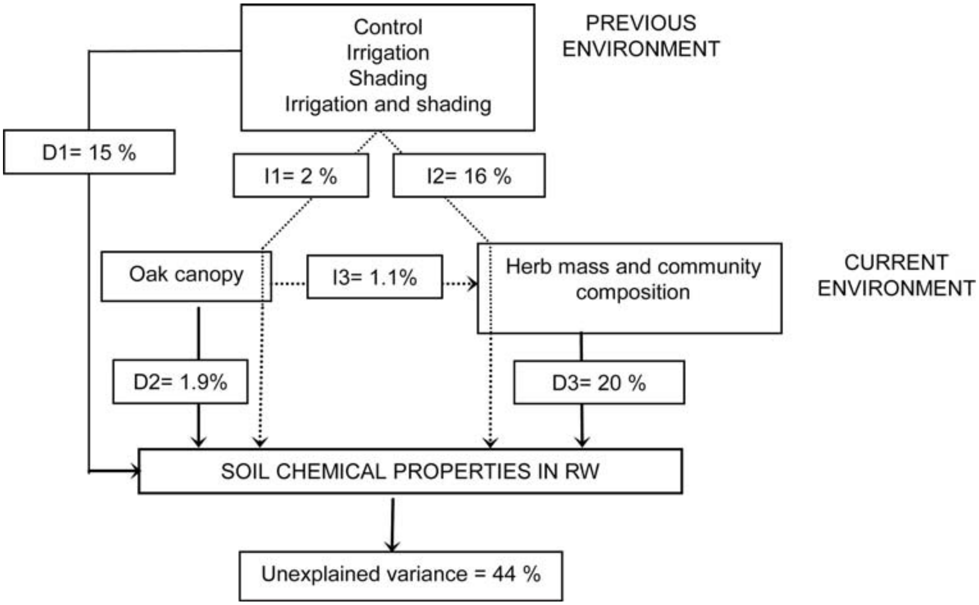

Figure 3. Contribution of direct effects (solid arrows) and indirect effects (dotted arrows) of previous and current environmental conditions to explain variance (56%) of soil chemical properties in the restored woodland plots (RW). D1: direct effects of previous management practices (control, irrigation, shading, and irrigation and shading); D2: direct effects of current oak canopy; D3 direct effects of current herb community (herb mass and species composition); I1 and I2: indirect effects of previous management practices through their effect on oak canopy and herb community, respectively; and I3: indirect effect of oak canopy through its effect on herb community.

Differences in soil conditions among the land use types studied here, specifically among the mature woodland and the three other land use types, may have affected our results to some extent. Since natural oak woodlands on productive soils in the region have completely been extirpated since long time ago for agriculture, the reference mature oak woodlands remain in more shallow and less productive soils.

According to our first hypothesis, concentration of P_2O_5 and K in the old-field and the restored woodland plots occupied intermediate positions along the soil chemistry gradient from active cropland to mature woodland, but concentration of C and total N did not follow the predicted pattern. Soil pH ranked active cropland > old field = restored woodland > mature woodland, although the only statistically significant difference was found for the active cropland-mature woodland comparison. One explanation for highest pH values in active cropland is cropland liming (Compton and Boone 2000; Smal and Olszewska 2008). Mature woodland showed higher concentration of C than the other three land use types, in agreement with previous studies (Compton and Boone 2000; Falkengren-Grerup *et al*. 2006). In active cropland most of the produced biomass in the arable is continuously extracted out from it, resulting in a reduction of the concentration of organic matter. Furthermore, tillage aerates the soil and breaks up organic residues, thus stimulating microbial activity and increasing soil organic matter decomposition (Brady and Weil 1999). Soil C did not differ among active cropland, old field and restored woodland sites, probably because C accumulation in soils after crop abandonment is slow in Mediterranean environments (Berg *et al*. 1993; Couteaux *et al*. 1995). Furthermore, as in restored woodland the roots of growing trees penetrate and concentrate C into deeper soil layers, limiting our soil sampling to the superficial 20 cm could not be adequate to observe any differences in C content (Jobbagy and Jackson 2000).

Unlike C content, nitrogen, phosphorous and potassium are commonly added nutrients to cropland. We did not find differences in total soil N concentration in the four studied habitat types. Some studies have reported increases in total soil N after agriculture abandonment and vegetation recovery (Alriksson and Olsson 1995; Côté *et al*. 2000) whereas others have failed to detect such increases (Camill *et al*. 2004; Marcos *et al*. 2007), as in this study. Nitrogen addition to croplands increases total N content in the agroecosystem and may thus attenuate the differences with succesional stages after field abandonment, where new build-up of organic matter with high C:N ratio slowly increases soil N content. As expected, active cropland had the highest concentration of P_2O_5 and K whereas mature woodland had the lowest. Agricultural use increased the concentration of P_2O_5 and K in the soil probably due to application of organic and inorganic fertilizers and this effect usually persist long time after crop abandonment (Compton and Boone 2000; Smal and Olszewska 2008) After restoration, concentration of P_2O_5 and K decreased probably due to uptake of these nutrients by plants.

Secondary succession versus *active woodland restoration*

Fourteen years after restoration beginning, vegetal structure in the old-field and in the restored woodland was different. Old-field was colonised by a dense herbaceous community where any woody species was established, which agree with that observed by other authors (Bonet and Pausas 2004). Woody vegetation recover in Mediterranean old-fields is usually hindered by summer drought and herbaceous competition (Rey Benayas 2005; Vallejo *et al.* 2006). Reforestation in the restored woodland created a relatively closed oak canopy, which reduced herbaceous cover and biomass, probably because water, nutrient and light deprivation (Ludwig *et al*. 2004; Pecot *et al*. 2007). Herbaceous gild had different cover in both restoration scenarios. Thus, whereas the legume and graminoid covers were similar in the old-field and in the restored woodland, forb cover was higher in the former. Differences in vegetation structure and composition between the old-field and the restored woodland could contribute to the differences observed in the soil chemical properties (Vinton and Burke 1995; Cornelissen *et al.* 1999; Cornwell *et al*. 2008; Guo *et al*. 2008).

Old-field and restored woodland plots showed similar amounts of C, K, P_2O_5, total N, NO^{3-}-N, total mineral N, and PO_4^{3-}-P availability. However, soils of the restored woodland plots displayed higher concentrations of NH^{4+}-N and higher availability of mineral N than old-field plots, partially supporting our second hypothesis. Thus, active restoration of vegetation after 13 years can contribute to increase only some variables related to soil fertility in the studied Mediterranean system. Other studies in a variety of ecosystems of the world, particularly in temperate prairies and forests, have reported restoration of soil fertility after cropland abandonment after a few decades (see revision in Rey Benayas *et al.* 2009).

Potential rates of mineralization were very low, or even negative, indicating a high potential of the plants and soil biota for immobilizing N in our study (Gallardo and Merino 1998). The lack of differences in ammonification, nitrification and mineralization rates between old-field and restored woodland soils can again be explained by the relatively short time since cropland abandonment in a relatively low productive environment. Changes in litter quality due to different plant composition may require long time to modify mineralization rates, since decomposition and incorporation of organic matter into the soil is usually slow (Berendse *et al.* 1989), particularly in Mediterranean environments (Berg *et al*. 1993; Couteaux *et al*. 1995). Moreover, it is important to consider that mineralization rates were measured at standard environmental conditions in the laboratory and, consequently, indirect effects of large plants on microclimatic conditions were omitted. Actual mineralization rates in the field could be different between old-field and restored woodland.

The amount of mineral N in the soil mainly depends on the balance between rates of mineralization and immobilization (Killham 1994; Accoe *et al*. 2004). Since nitrogen mineralization was similar in old-field and in restored woodland plots, the lower amount of NH^{4+}-N and the lower availability of mineral N found in the old-field suggest that immobilization of N was greater in this habitat than in restored woodland. This means that N uptake by plants and /or soil microorganism exceeds the rate at which N is released through decomposition of organic matter. Fast growing species such as herbs are more nutrient demanding than slow growing holm oak and therefore have greater potential to uptake nutrients (Poorter *et al*. 1990), which may explain the lower concentration of NH^{4+}-N in old-field plots. Furthermore, previous studies demonstrated that N immobilization in the soil microbial biomass is higher in grassland than in forest soils (Davidson *et al*. 1990; Davidson *et al*. 1992; Hart *et al*. 1993), which can explain the higher availability of soil mineral N in restored woodland. Differences in cover of guilds in the herbaceous community between the old-field and the restored woodland could also explain differences in soil chemistry between both land use types (Tilman *et al*. 1997). Legumes usually increase N availability in the soil, because of N_2 fixation and higher N input via litter decomposition, whereas grasses and forbs reduce it, generally due to their high root production (Hooper and Vitousek 1998; Oelmann *et al.* 2007; Davies *et al*. 2009). In our study, there were no differences in legume and graminoid cover between the old-field and the restored woodland, but forb cover was higher in the old-field, which could also contribute to the lower NH^{4+}-N concentration and N availability in this restoration scenario.

Carryover effects of restoration management

In agreement with our third hypothesis, soil chemical properties in the studied restored woodland reflected both carryover effects of previous restoration management (D1 in Fig. 3), particularly irrigation, and effects of current environmental conditions that were mostly related to herbaceous biomass and herb community composition (D3 in Fig. 3). Irrigation practices had an effect on pH, the concentrations of P_2O_5 and K, and on the availability of mineral N that can be noticeable more than 10 years after being effected. This can be explained by the mitigation of water stress on soil organisms that stimulate organic mater decomposition, since soil moisture is a major environmental factor controlling organic matter decomposition and soil fertility in Mediterranean ecosystems (Couteaux *et al*. 1995). Soil chemical properties were also indirectly affected by previous management practices mainly through its influence on the herbaceous community (I2 in Fig. 3), since indirect effects through oak canopy (I1 in Fig. 3) were low. It appears that the restoration techniques applied determined the structure and composition of the herb community (Cayuela *et al*. 2008), resulting in litter quality and quantity differences that may have influenced the measured soil properties (Vinton and Burke 1995). Thus, the higher legume cover found in irrigated plots may explain the higher availability of N in these plots.

The relative effects of oak canopy, the other component of current environmental conditions, on soil chemical properties (D2 in Fig. 3) were small as compared to the effects of the herbaceous community (D3 in Fig. 3). Oak litter has a high lignin and tannin content (Allen *et al*. 1974), which results into slower decomposition rates and incorporation of nutrients into the soil (Gallardo and Merino 1993; Couteaux *et al*. 1995; Satti *et al*. 2003). Finally, unexplained variation found in this study may be due to a combination of stochastic processes and mechanisms not accounted for related to microclimate, regional variability, oak root development, soil fauna, and others (Davidson *et al*. 1992; Couteaux *et al*. 1995; Aerts and Chapin 2000).

Conclusions

This study provides evidence of the overall slow dynamics of soil chemical properties in restored Mediterranean ecosystems after cropland abandonment. As a result, we observed relatively little variation of soil properties in restored abandoned cropland over a time span of 13 years. Yet experimental evidence allows us to highlight that active restoration of native woodland vegetation can speed up the recovery of soil properties in comparison with secondary succession, and take advantage of management techniques used to facilitate early establishment of introduced seedlings.

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Viaja por el mundo,
viaja entre los mundos,
puedes ir caminando,
brincando o incluso corriendo,
tú eliges, pero nunca olvides
pararte a oler las flores de tú camino

Marta Rueda García

Capítulo 7

Capítulo 7

Discusión general

En esta Memoria de Tesis Doctoral se han desarrollado diversas ideas encaminadas a mejorar la revegetación forestal de campos agrícolas abandonados en ambientes mediterráneos y comprender algunos procesos subyacentes a dicha revegetación. En el presente capítulo de Discusión se integran los resultados de cada uno de los objetivos específicos planteados en la Introducción general para extraer las conclusiones de la Tesis.

¿Por qué los fenotipos *productivos* tienden a establecerse mejor que los fenotipos *xeromorfos* en las plantaciones forestales mediterráneas?

Los fenotipos *productivos* (plantas de gran porte y elevado contenido de N) de *Pinus halepensis* presentaron mayor supervivencia y crecimiento que los fenotipos *xeromorfos* (plantas de porte pequeño y bajo contenido de N) (**capítulos 2** y **3**). Resultados semejantes también se han obtenido para diversas especies en ambientes mediterráneos sub-húmedos y secos (Cortina *et al*. 1997; Puértolas *et al.* 2003; Villar-Salvador *et al*. 2004; Tsakaldimi *et al*. 2005; Navarro *et al.* 2006b; Del Campo García *et al*. 2007; Villar-Salvador *et al.* 2008) mediterráneos semiáridos (Luis *et al*. 2009; Oliet *et al*. 2009) y en ecosistemas templado-húmedos y boreales (Lamhamedi *et al.* 1998; Noland *et al*. 2001; South *et al*. 2005). Una contribución importante de esta Tesis ha sido esclarecer algunos de los mecanismos ecofisiológicos implicados en este patrón. Los resultados obtenidos se sintetizan en el modelo conceptual recogido en la Fig.1.

La supervivencia de los plantones en las repoblaciones está ligada a la producción de raíces nuevas después del transplante (Ritchie y Dunlap 1980; Burdett *et al*. 1983; Burdett 1990; Grossnickle 2005), ya que aseguran su abastecimiento de agua y nutrientes (Grossnickle 2005; Padilla y Pugnaire 2007). Este requisito es especialmente importante en ambientes con periodos secos largos, como los ecosistemas mediterráneos (Grossnickle 2005; Padilla y Pugnaire 2007). La formación de nuevas raíces debe ocurrir durante el período húmedo del año, cuando el suelo dispone de suficiente humedad para garantizar la expansión celular asociada al crecimiento de los órganos. El desarrollo radical en especies forestales mediterráneas perennifolias depende en buena medida de la fotosíntesis de la planta (Moreno, 2003; M. Uscola comunicación personal). Las plantas grandes, por disponer de más follaje y mayor tasa neta de fotosíntesis, poseen mayor capacidad fotosintética que las plantas pequeñas (**capítulos 2** y **3**), por lo que potencialmente disponen de más carbohidratos para el crecimiento aéreo y radical y para el mantenimiento general del metabolismo basal (Fig. 1). Un sistema radical bien desarrollado puede incrementar la absorción de agua y nutrientes, lo que incrementa la conductancia estomática y, por tanto, la tasa de fotosíntesis. En nuestro caso, el mayor desarrollo radical no se tradujo en una mejora del estado hídrico de la planta (**capítulos 2** y **3**), tal como se ha observado en otros estudios (Bellot *et al*. 2002; Padilla y Pugnaire 2007), sino que probablemente aumentó la fotosíntesis por medio de un incremento de la conductividad hidráulica específica de la planta (Grossnickle y Russell 1990; Hubbard *et al*. 2001; Sayer *et al.* 2005).

La concentración de N en una planta puede también determinar el crecimiento y la supervivencia post-transplante (Fig. 1, **capítulos 2** y **3**). La concentración de N en los tejidos y el tamaño de la planta determinan su contenido de N y, por tanto, la cantidad potencial de N removilizable (Millard 1996; Salifu y Timmer 2003; Silla y Escudero 2003). En términos absolutos, las plantas grandes removilizaron más N que las pequeñas, y entre las plantas pequeñas removilizaron más N las de mayor concentración de este nutriente. Una aportación importante de esta Tesis fue demostrar que las plantas con alta concentración de N, independientemente de su tamaño, removilizaron en términos relativos más N que las plantas con baja concentración de N (**capítulo 2**). Esto podría incrementar la eficiencia en el uso del N almacenado de las plantas con gran concentración de N en ambientes pobres en nutrientes. Por otro lado, las plantas grandes son capaces de competir mejor con otras plantas porque también tienen mayor capacidad de absorción de N.

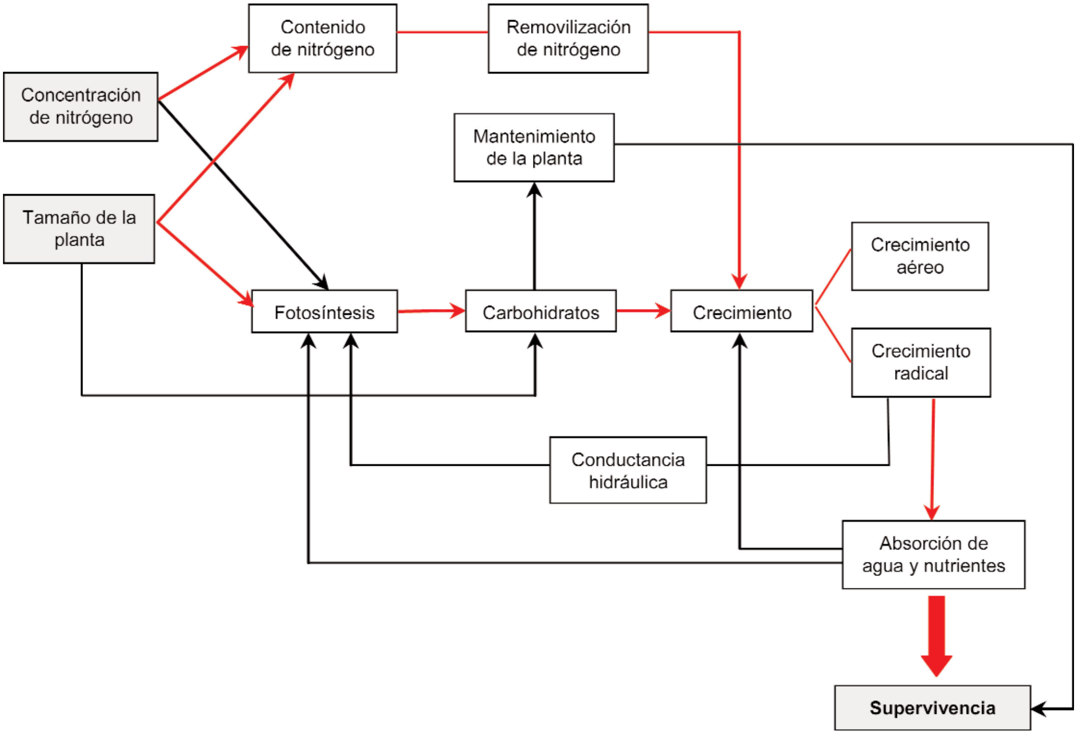

Figura 1. Modelo conceptual de los mecanismos que relacionan el tamaño y la concentración de N de los plantones al salir del vivero con su supervivencia y crecimiento posterior en campo. En rojo se muestran los procesos demostrados en esta Tesis Doctoral, y en negro los procesos que faltan por demostrar o que no se han abordado en este trabajo.

El tamaño de los plantones tiene un papel más importante en el establecimiento post-transplante y funcionamiento que la concentración de N en los tejidos en *P. halepensis* (**capítulos 2** y **3**). Esta afirmación se sustenta en el hecho de que, a igual concentración de N, las plantas más grandes tuvieron mayor supervivencia, intercambio gaseoso y crecimiento que las plantas pequeñas. Por el contrario, al comparar plantas de tamaño similar pero marcadas diferencias de concentración de N, no se observaron diferencias de supervivencia, intercambio gaseoso y crecimiento aéreo (**capítulo 2**), aunque las plantas con mayor concentración de N presentaron mayor crecimiento radical que las plantas con baja concentración de N (**capítulo 3**). La presencia de un cuarto tipo de fenotipo (plantas grandes con baja concentración de N) en el capítulo 2 nos hubiese permitido apoyar con mayor seguridad esta conclusión. Aunque experimentalmente no fue posible producir este fenotipo, creemos que nuestra conclusión es sólida; es decir, es razonable asumir una relación lineal de la respuesta en el campo de los plantones con su tamaño y concentración de N, tal y como indican los resultados del **capítulo 3**. En los **capítulos 2** y **3**, las diferencias de tamaño entre los plantones ensayados fueron mayores que las diferencias en la concentración de N, lo que puede explicar por qué el establecimiento dependió más del tamaño de las plantas que de su concentración de N. El tamaño de la planta suele ser más plástico que la concentración de N en respuesta a los tratamientos de cultivo en vivero (Villar-Salvador *et al*. 2004; Oliet *et al.* 2009), por lo que pensamos que la mayor influencia relativa del tamaño en relación con la concentración de N de la planta sobre el establecimiento de los plantones de una savia de *P. halepensis* no se restringe a nuestras condiciones experimentales.

La capacidad de atender a varias funciones básicas (crecimiento, mantenimiento, metabolismo secundario y reproducción) depende no sólo de la especie considerada. Esta Tesis evidencia que también depende de las características funcionales de los individuos a nivel intraespecífico. Los individuos grandes de una savia de *P. halepensis* fueron capaces de atender a la vez su crecimiento aéreo y radical sin interferencia mutua, mientras que ambos crecimientos interfirieron en los individuos pequeños (**capítulo 3**). Al no poder compaginar el crecimiento aéreo con el radical, es probable que las plantas pequeñas reduzcan su crecimiento radical al comenzar su crecimiento aéreo, lo que podría comprometer su supervivencia durante el periodo seco si el sistema radical no se ha desarrollado suficientemente. Pensamos que las plantas grandes pueden mantener el crecimiento de ambas partes simultáneamente porque presentan mayor capacidad fotosintética y/o porque tienen mayor contenido de N para su removilización (Malik y Timmer 1996; Millard 1996; Salifu y Timmer 2003). La concentración de N en los tejidos no afectó a la interferencia entre el crecimiento aéreo y el radical, por lo que la capacidad de las plantas grandes para simultanear el crecimiento aéreo y el radical parece estar ligada solamente a una mayor capacidad fotosintética.

En contraste con lo observado en *P. halepensis,* en individuos de *Q. ilex* de 14 años de edad no se encontraron compromisos de asignación de recursos entre el crecimiento aéreo y el radical, pero sí entre el crecimiento y la reproducción (**capítulo 5**). Además, los individuos reproductores crecieron menos que los individuos no reproductores, aunque estas diferencias solamente se observaron en individuos grandes, cuyo volumen de copa fue superior a los 2 m^3 (Fig. 2). Al aumentar el tamaño de los individuos se incrementan los costes de mantenimiento, por lo que puede aparecer competencia por los recursos entre las funciones de crecimiento y reproducción (Obeso 1997; Greer y McCarthy 2000; Wheelwright y Logal 2004). En individuos pequeños, sin embargo, los costes asociados al mantenimiento son menores, por lo que el crecimiento y la reproducción podrían simultanearse sin interferencia.

Los resultados obtenidos con *P. halepensis* (**capítulo 3**) y con *Q. ilex* (**capítulo 5**) sugieren que la influencia del tamaño de los individuos en los compromisos de asignación de recursos entre distintos órganos o funciones puede variar con la ontogenia y/o diferir entre especies.

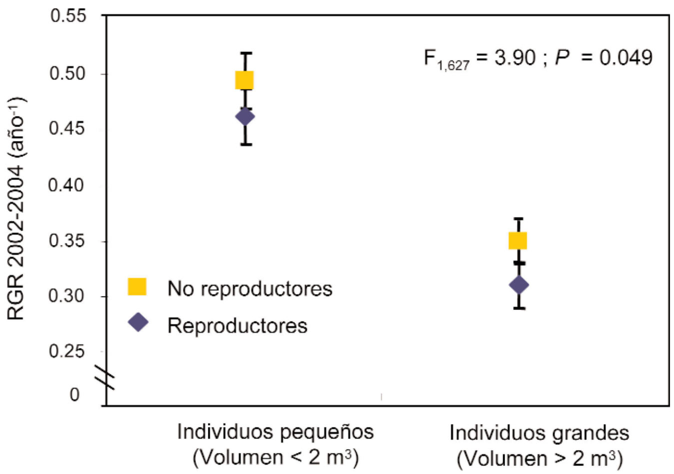

Figura 2. Tasa de crecimiento relativo en el período 2002-2004 (RGR 2002-2004) en individuos reproductores y no reproductores de *Q. ilex* en función del volumen de su copa. Los datos son medias ± 1 ES.

Las diferencias de establecimiento entre fenotipos dependen del ambiente post-transplante

El efecto del tamaño y concentración de N en el desarrollo de los plantones en el campo depende del nivel de estrés al que los plantones están sometidos después de la plantación. Nuestros resultados demuestran que los atributos funcionales tuvieron mayor relevancia al incrementarse el estrés durante el establecimiento. En contra de la hipótesis inicial formulada en introducción general de esta Tesis (Fig. 3 del capítulo 1), los fenotipos *productivos* no presentaron peor desarrollo en campo que los fenotipos *xeromorfos* en condiciones de estrés hídrico severo, si no que fue similar o incluso mejor. Pensamos que ello será así siempre que los fenotipos *productivos* dispongan tiempo suficiente y condiciones iniciales de humedad edáfica para arraigar, lo que indica la importancia de elegir bien la fecha de plantación. En condiciones de bajo estrés hídrico, ambos fenotipos presentaron tasas de supervivencia similares (Fig. 3). Al aumentar el estrés y alcanzarse niveles moderados de estrés hídrico, como ocurrió al final de la primavera en competencia con las hierbas (**capítulo 2**), o en los claros en la parcela de La Mancha (**capítulo 4**), los fenotipos *productivos* presentaron mayor supervivencia que los fenotipos *xeromorfos*. Finalmente, en ambientes de estrés hídrico severo, como en verano en presencia de hierbas en la parcela de Guadalajara (**capítulo 2**), o en los claros con o sin hierbas en la parcela de La Mancha (**capítulo 4**), la supervivencia de los fenotipos *productivos* fue similar o mayor que la de los fenotipos *xeromorfos*. En cuanto al crecimiento, en la parcela de Guadalajara las plantas grandes crecieron más que las pequeñas independientemente del estrés post-plantación, si bien la diferencia fue mayor en condiciones de bajo estrés (**capítulo 2**). En la parcela de La Mancha (**capítulo 4**), sin embargo, no hubo diferencias de crecimiento entre fenotipos de plantas.

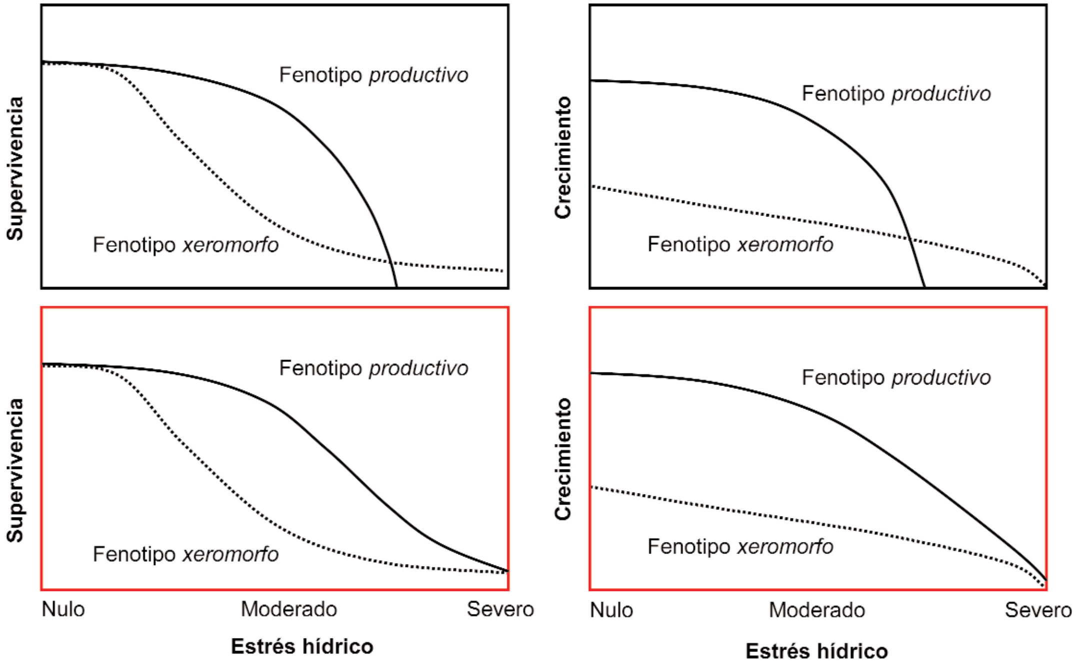

Figura 3. Hipótesis de trabajo (arriba-cuadros negros) y resultados obtenidos (abajo-cuadros rojos) relativos a la influencia que tienen las características morfológicas de las plantas en su supervivencia y crecimiento en función del nivel de estrés hídrico del lugar de plantación. Fenotipo *productivo* hace referencia a plantas grandes con una proporción entre la masa aérea y radical y una concentración de nutrientes elevada dentro del rango habitual de la especie. Fenotipo *xeromorfo* hace referencia a plantas con los atributos funcionales contrarios.

Facilitación directa e indirecta en retamares mediterráneos

Retama sphaerocarpa (retama) facilita el establecimiento de *Q. ilex*, tanto de forma directa reduciendo el estrés hídrico y la fotoinhibición de las plantones, como de forma indirecta reduciendo la capacidad competitiva de la comunidad herbácea que se desarrolla bajo su dosel (**capítulo 4**).

Las interacciones de facilitación y competencia entre plantas ocurren de forma simultánea, y el resultado neto de las mismas depende de las condiciones bióticas y abióticas del ambiente, las especies involucradas y la fase del ciclo vital (Callaway 2007). El **capítulo 4** va más allá y demuestra que 1) no sólo el balance competencia-facilitación varía con las condiciones ambientales, sino que el balance facilitación directa-facilitación indirecta también lo hace, y 2) el resultado del balance competencia-facilitación no sólo depende de las especies consideradas, sino también de las características funcionales de los individuos facilitados a nivel intraespecífico.

El tipo de facilitación predominante parece depender de las condiciones climáticas del año (**capítulo 4**), prevaleciendo la facilitación indirecta en los años de primaveras secas cuando se establece una intensa competencia entre el arbusto nodriza y la comunidad herbácea, lo que favorece indirectamente el establecimiento de *Q. ilex*. Por el contrario, la importancia relativa de la facilitación directa, mediada por la suavización de las condiciones microclimáticas, parece aumentar con la aridez estival. Así, no solo la sequía estival, sino también las condiciones climáticas primaverales pueden determinar las interacciones de facilitación.

No todos los individuos dentro de una misma especie responden de la misma forma a la facilitación (**capítulo 4**). La retama facilitó el establecimiento de las plantas pequeñas, mientras que las plantas más grandes sobrevivieron de forma similar dentro y fuera de su dosel. Una importante consecuencia de este resultado es que si el tamaño de los plantones tiene una base genética (Leiva y Fernández-Alés 1998), la presencia de arbustos nodriza podría contribuir a mantener la diversidad genética de las poblaciones de encina.

El uso de plantas nodrizas como técnica de revegetación

La utilización de las plantas nodriza como técnica de restauración de la vegetación se considera una alternativa a otras prácticas de revegetación de mayor costo económico y ambiental inicial (Maestre *et al*. 2001; Castro *et al*. 2004; Gómez-Aparicio *et al*. 2004; Padilla y Pugnaire 2006). El aumento de la superficie ocupada por especies arbustivas en campos agrícolas abandonados en ambientes mediterráneos, bien de forma natural o porque han sido introducidas en proyectos de reforestación, incrementa el atractivo de esta opción. Es importante, sin embargo, destacar las bajas tasas de supervivencia de *Q. ilex* encontradas bajo *R. sphaerocarpa* (17 y 23% en 2006 y 2007, respectivamente, **capítulo 4**), que coinciden con las encontradas para otras especies leñosas en ambientes mediterráneos (Maestre *et al*. 2001; Castro *et al.* 2004). Para determinados objetivos de restauración de la vegetación, las mejoras de supervivencia por las plantas nodriza no garantizan una recuperación suficiente de la vegetación madura. Así, si el fin es la recuperación de la masa forestal a corto-medio plazo, por ejemplo para evitar la erosión o para el aprovechamiento maderero o de frutos, el empleo de plantas nodriza no parece ser una herramienta viable. Por el contrario, si la revegetación persigue la introducción de algunos individuos de especies tardías en la sucesión para acelerar la sucesión secundaria o conservar poblaciones de ciertas especies leñosas amenazadas y la obtención de rentas no es una prioridad, el uso de plantas nodrizas es una técnica válida.

Restauración de las propiedades y procesos ecosistémicos: efectos de las condiciones ambientales pasadas y presentes

Las condiciones edáficas pueden considerarse un síndrome de multitud de procesos que operan a escala de ecosistema. En ambientes mediterráneos, la restauración de las propiedades edáficas es un proceso muy lento. Después de 13 años, tanto la restauración activa como la restauración pasiva (sucesión secundaria) de la vegetación afectaron poco a las propiedades químicas del suelo de un campo agrícola abandonado (**capítulo 6**). La concentración de macronutrientes, pH y la tasa de mineralización de N fueron similares en las parcelas restauradas de forma pasiva y de forma activa. La descomposición de la materia orgánica es un proceso muy lento (Berendse *et al*. 1989), especialmente en estos ecosistemas (Berg *et al*. 1993; Couteaux *et al*. 1995), lo que podría explicar por qué después de 13 años las diferencias en la composición química de la hojarasca propia de cada tipo de restauración no se han traducido en cambios en las tasas de mineralización ni en otras propiedades. No obstante, se encontraron diferencias en la concentración de amonio y la disponibilidad de N mineral en el suelo. Ello podría explicarse por mayores tasas de inmovilización de N en la microfauna edáfica en las parcelas restauradas de forma activa (Davidson *et al.* 1990; Davidson *et al*. 1992; Hart *et al.* 1993) y por una mayor demanda de nutrientes en estas parcelas (Poorter *et al.* 1990).

Las condiciones ambientales, tanto pasadas como presentes, determinan el funcionamiento de los ecosistemas (Metcalfe y Monaghan 2001; Relyea 2002; Cayuela *et al*. 2008). Por ello, las propiedades químicas del suelo estudiadas en este trabajo reflejaron tanto la influencia del ambiente previo como de las condiciones ambientales actuales (**capítulo 6**). Los tratamientos de manejo aplicados durante el establecimiento de los plantones de *Q. ilex* influyeron en la composición química del suelo tanto de forma directa, probablemente mediante la estimulación de la descomposición y mineralización de la materia orgánica, como de forma indirecta, principalmente por su efecto en la composición y estructura de la comunidad herbácea (Cayuela *et al.* 2008). Las condiciones ambientales actuales, especialmente la comunidad herbácea, también influyeron en las propiedades químicas edáficas. El pequeño efecto del dosel de encinas puede deberse a la baja calidad de su hojarasca, que ralentiza su descomposición y mineralización, requiriendo, por tanto, de más tiempo para poder modificar las propiedades edáficas.

De forma similar, el funcionamiento de individuos de *Q. ilex* de 14 años de edad no es sólo consecuencia de las condiciones ambientales presentes sino también de las del pasado (**capítulo 5**). Una vez interrumpidos los tratamientos de riego y sombra artificial aplicados durante su establecimiento, los individuos respondieron de forma diferente según el tratamiento aplicado; además, las respuestas fueron diferentes según el rasgo de vida analizado. El manejo previo no afectó a la mortalidad futura de los plantones, pero disminuyó su crecimiento, redujo el desarrollo del sistema radical lo que incrementó la relación parte aérea/parte radical, aceleró la madurez sexual y aumentó el esfuerzo de producción de bellotas.

Consideraciones adicionales y líneas futuras de investigación

Como se ha indicado anteriormente, aún quedan por investigar aspectos relacionados con las características funcionales de las plantas que también podrían determinar la ventaja de los fenotipos *productivos* sobre los *xeromorfos*. Uno de ellos serían las diferencias de costes de mantenimiento entre fenotipos. En plantas cultivadas en contendor, las más grandes siempre tienden a tener mayor proporción de parte aérea/parte radical que las plantas pequeñas porque el contenedor constriñe más el crecimiento radical que el aéreo. Ello puede implicar menores costes de mantenimiento de la planta y, por tanto, mayor disponibilidad de carbohidratos para otras funciones. En este sentido, merece ser estudiado el papel de los carbohidratos de reserva en la capacidad de supervivencia en situaciones de estrés severas en las que el balance de carbono de la planta se hace negativo. Pensamos que los carbohidratos de reserva serán importantes para la supervivencia en aquellas especies que siguen una estrategia de ahorro de agua por cierre de estomas con potenciales hídricos elevados como los pinos. También merece ser estudiada la diferencia de conductividad hidráulica específica entre fenotipos como factor que determina las diferencias de su capacidad fotosintética. Se han hecho algunos trabajos que relacionan la conductividad hidráulica específica con la fertilización (Trubat *et al*. 2006), pero son necesarios trabajos que analicen la conexión entre la conductividad hidráulica específica con la fotosíntesis y su relación con el tamaño de la planta.

Tampoco se conoce bien cuál es el tamaño óptimo que deben tener los plantones de las distintas especies forestales mediterráneas para asegurar su establecimiento. Trabajos futuros deberían definir cuál es el tamaño de planta más apropiado para asegurar el éxito de las repoblaciones según la especie y el ambiente de plantación. Los rangos de tamaño de planta contemplados en la legislación actual sobre calidad exterior de planta pueden no ser realistas ni ajustarse bien al tamaño necesario para un buen desarrollo en el campo, especialmente teniendo en cuenta la diversidad de ambientes que algunas especies forestales pueden ocupar. Así, algunos autores proponen estrechar los rangos de tamaño de planta reconocidos en la legislación vigente, preponderando plantas de mayor tamaño (Navarro *et al*. 2006a).

Finalmente, en relación con la facilitación, son necesarias más investigaciones que analicen la incidencia de la facilitación indirecta entre plantas en ecosistemas mediterráneos, al igual que experimentos diseñados específicamente para demostrar bajo qué condiciones ambientales la facilitación indirecta es relevante.

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Margarita Huerta Silva

Capítulo 8

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Conclusiones generales

1. Los fenotipos *productivos* de *Pinus halepensis* presentaron un mejor establecimiento que los fenotipos *xeromorfos*, mientras que las diferencias entre los fenotipos de *Quercus ilex* fueron muy pequeñas. La mayor supervivencia y crecimiento de los fenotipos *productivos* en *P. halepensis* se asoció a un mayor intercambio gaseoso, crecimiento radical y uso del nitrógeno que en los fenotipos *xeromorfos*, pero no a una mejora del estado hídrico.

2. El efecto del tamaño y la concentración de nitrógeno en el establecimiento de los plantones en campo dependió del nivel de estrés al que los plantones están sometidos después de la plantación. Cuando el estrés ambiental fue reducido no se observaron diferencias entre fenotipos de *P. halepensis* y *Q. ilex*. Cuando el estrés fue moderado los fenotipos *productivos* se establecieron mejor que los fenotipos *xeromorfos*. Finalmente, cuando el estrés fue severo el establecimiento de los fenotipos *productivos* fue mejor o similar que el de los fenotipos *xeromorfos*.

3. El tamaño de los plantones en *P. halepensis* tuvo una mayor influencia en el establecimiento en el campo que la concentración de nitrógeno en los tejidos.

4. La capacidad de los individuos de *P. halepensis* y *Q. ilex* para atender simultáneamente dos o más funciones básicas dependió de su tamaño. Los plantones de gran tamaño de *P. halepensis* fueron capaces de atender simultáneamente el crecimiento aéreo y el radical, mientras que los individuos pequeños no pudieron atender esta doble función. En individuos de *Q. ilex* de 14 años de edad, no se encontraron compromisos de asignación de recursos entre el crecimiento aéreo y el radical, pero sí entre el crecimiento y la producción de bellotas, aunque esto sólo ocurrió en los individuos relativamente grandes, con dosel mayor de 2 m^3.

5. Tanto la facilitación directa como la indirecta fueron mecanismos importantes para el establecimiento de *Q. ilex* en los retamares y su importancia relativa varió con las condiciones climáticas de los años estudiados. La facilitación indirecta, basada en la disminución de la competencia herbácea con los plantones facilitados, fue importante en el año de primavera seca cuando la competencia entre el arbusto nodriza y las hierbas fue intensa. En cambio la facilitación directa, debida a la mitigación de las condiciones microclimáticas adversas, aumentó con la aridez estival.

6. El resultado de la facilitación dependió del tamaño de los individuos de *Q. ilex*. *Retama sphaerocarpa* facilitó el establecimiento de los plantones pequeños, mientras que los plantones más grandes no fueron afectados por la presencia de ésta. Estas evidencias apuntan a que el resultado de la facilitación entre plantas puede depender de las características funcionales de los individuos facilitados a nivel intraespecífico.

7. Si el fin de la revegetación es la recuperación de la masa forestal a corto-medio plazo con densidades elevadas de individuos, la utilización de plantas nodriza no parece ser una técnica viable debido a las bajas tasas de supervivencia de las plantas facilitadas bajo su dosel. Por el contrario, si la revegetación persigue acelerar la sucesión secundaria, el empleo de plantas nodriza puede ser una técnica valiosa.

8. Después de 13 años, tanto la restauración activa de la vegetación como la pasiva (sucesión secundaria) modificaron poco las propiedades químicas del suelo de un campo agrícola abandonado. La mayor diferencia entre estos dos tipos de restauración fue un incremento de la concentración de amonio y disponibilidad de nitrógeno para las plantas en las parcelas restauradas de forma activa.

9. Tanto el crecimiento y la producción de bellotas de los individuos de *Q. ilex* de 14 años de edad como las propiedades edáficas en el momento presente estuvieron influenciadas positivamente por el manejo con riego estival y sombra artificial aplicado durante el establecimiento inicial de la plantación.