El conocimiento de las interacciones que se establecen entre las plantas y los suelos sobre los que se desarrollan es esencial para comprender la dinámica de los ecosistemas y poder prever su respuesta ante el cambio global. En esta tesis doctoral se aborda el estudio de las interacciones ecológicas entre el suelo y las especies forestales de los bosques mediterráneos con el objetivo de conocer lo mecanismos subyacentes a estas interacciones, elucidar posibles procesos de retroalimentación y comprender cómo estos dos componentes gobiernan conjuntamente el funcionamiento del ecosistema.

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Interacciones planta-suelo en un bosque mediterráneo

C. Aponte

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Cristina Aponte Perales
Tesis doctoral
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Interacciones planta-suelo en un bosque mediterráneo

CRISTINA APONTE PERALES

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

Sevilla, Octubre de 2010

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HACEN CONSTAR:

Que el trabajo descrito en la presente memoria, titulado: ¨**Interacciones planta-suelo en un bosque mediterráneo**¨ ha sido realizado bajo su dirección por Dña. Cristina Aponte Perales en el Instituto de Recursos Naturales y Agrobiología de Sevilla, CSIC, y reúne todos los requisitos necesarios para su aprobación como Tesis Doctoral.

Sevilla, 16 de octubre de 2010

Dr. Teodoro Marañón Arana **Dr. Luis Ventura García Fernández**

Mª Cruz Díaz Antunez Barradas, Profesora Titular de la Universidad de Sevilla

HACE CONSTAR:

Que el trabajo descrito en la presente memoria, titulado: **“Interacciones planta-suelo en un bosque mediterráneo”** ha sido realizado bajo su tutela por Dña. Cristina Aponte Perales dentro del Programa de Doctorado de Biología de la Conservación, del Departamento de Biología Vegetal y Ecología de la Universidad de Sevilla.

Sevilla, 16 de octubre de 2010

Mari Cruz Diaz Barradas

Dr. Mª Cruz Díaz Antunes Barradas

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Resumen

El conocimiento de las interacciones que se establecen entre las plantas y los suelos sobre los que se desarrollan es esencial para comprender el funcionamiento de los ecosistemas y poder prever su respuesta ante el cambio global. En esta tesis doctoral se aborda el estudio de las interacciones ecológicas entre el suelo y las especies forestales de los bosques mediterráneos con el objetivo de conocer los mecanismos subyacentes a estas interacciones, elucidar posibles procesos de retroalimentación y comprender cómo estos dos componentes gobiernan conjuntamente el funcionamiento del ecosistema. Para ello se realizaron trabajos descriptivos y experimentales en condiciones de campo. Los estudios se desarrollaron en el Parque Natural Los Alcornocales, situado en las provincias de Cádiz y Málaga. El Parque comprende un área aproximada de 170 000 ha y su vegetación está dominada por un bosque mixto de alcornoque (*Quercus suber* L.) y quejigo moruno (*Q. canariensis* Willd.).

En primer lugar se exploró el espectro de rasgos foliares en una comunidad de plantas leñosas y se investigaron las relaciones a nivel de especie entre los rasgos foliares y otros índices funcionales relacionados con la adquisición de recursos. Además se evaluó la variación de los rasgos foliares funcionales a nivel de comunidad y su relación con factores ambientales clave. Para ello se estudiaron diecisiete especies leñosas coexistentes incluyendo árboles, arbustos y enredaderas. Los rasgos foliares químicos, en particular el contenido de micronutrientes y elementos no esenciales, fueron más variables entre las especies que los rasgos morfológicos. El contenido isotópico foliar reflejó aspectos funcionales de las especies, como su eficiencia en el uso del agua o el tipo de interacciones simbióticas. La relación encontrada entre la masa foliar por unidad de área y múltiples variables funcionales y ambientales a nivel de especie y de comunidad señaló a esta característica foliar como un importante marcador funcional indicador de la estrategia de las especies en la adquisición de recursos, de su interacción con las condiciones ambientales y de la dinámica funcional de la comunidad.

A continuación, la tesis se centra en las dos especies de quercíneas que coexisten en estos bosques, la primera de hoja perenne y esclerófila (*Q. suber*) y la segunda de hoja marcescente y no-esclerófila (*Q. canariensis*), y en las relaciones que establecen con el suelo subyacente. Se investigó la influencia de las dos especies de quercíneas en las condiciones de fertilidad del suelo, para lo cual se estudiaron cinco componentes del sistema planta-suelo (hojas vivas, desfronde, hojarasca, suelo superficial y profundo). Se detectó un gradiente global de enriquecimiento en nutrientes común para los cinco componentes estudiados, a lo largo del cual se diferenciaron los nichos biogeoquímicos de las dos quercíneas. La especie marcescente *Q. canariensis* poseía tejidos más enriquecidos y ocupaba ambientes más fértiles que la perennifolia *Q. suber*. La

existencia de un posible ciclo de retroalimentación en el que las dos especies de quercíneas, a través de un distinto retorno de nutrientes, modificaban las propiedades del suelo de un modo tal que repercutía positivamente sobre la presencia de la propia especie se contrastó mediante modelos causales.

Seguidamente se estudiaron los mecanismos mediante los cuales ambas especies de quercíneas afectan al proceso de descomposición de la hojarasca y se investigaron fenómenos asociados a la descomposición en bosques mixtos. Se analizaron los patrones de inmovilización y liberación de nutrientes durante el proceso de descomposición de la hojarasca. Las especies influyeron en la dinámica de la descomposición tanto a través de la calidad de su desfronde como mediante las condiciones ambientales que generan. Los cambios en el contenido de calcio de la hojarasca y el suelo explicaron en un grado significativo las diferencias observadas en la dinámica de descomposición.

Para conocer la repercusión de los cambios abióticos en las comunidades bióticas del suelo se investigó el efecto que la estacionalidad, el tipo de cobertura vegetal, incluyendo la cobertura de alcornoque y de quejigo, y la profundidad del suelo tenían sobre el contenido en carbono, nitrógeno y fósforo microbiano de los suelos forestales mediterráneos estudiados. Se observaron cambios estacionales en el contenido microbiano de N y P, cuyos valores fueron superiores en primavera y otoño. Los valores registrados de las diferentes propiedades microbianas bajo cobertura de *Q. canariensis* y bajo matorral fueron mayores que bajo *Q. suber* y en zonas abiertas. Estas diferencias sólo fueron perceptibles en primavera. Los valores microbianos de C, N y P decrecieron significativamente con la profundidad en todos los casos estudiados.

Finalmente, se evaluó la diversidad y estructura de la comunidad de hongos ectomicorrícicos asociados a las raíces de *Q. canariensis* y *Q. suber* empleando técnicas moleculares para la identificación de especies. La comunidad de hongos simbiontes estaba dominada por tres familias: Telephoraceae, Russulaceae y Cortinariaceae. Se observó que la distribución de especies simbiontes respondía a las condiciones de fertilidad y acidez de suelo superficial generadas por ambas quercíneas. Este efecto indirecto de la especie huésped sobre la comunidad de hongos ectomicorrícicos se relacionó con cambios en la composición específica y en la diversidad filogenética de la comunidad micorrícica.

Abstract

The interactions established between plants and soil are essential to ecosystem functioning. To understand the mechanisms of these interactions is crucial to certainly predict ecosystem responses to global change. This PhD thesis aims to contribute to the knowledge of the ecological interactions between soil and plant species of the Mediterranean forests. The objectives of this study were to unveil the mechanisms underlying these interactions, to elucidate the possible feedback processes that exist between plants and soil communities and to understand how these two components –plant and soil- influence the ecosystem dynamics. For that purpose several descriptive and experimental studies were conducted in “Los Alcornocales” Natural Park, located in southern Spain. The park covers an approximate area of 170 000 ha and its vegetation is dominated by a mixed forest of cork oak (*Quercus suber* L.) and Algerian oak (*Q. canariensis* Willd.).

First, we studied the spectrum of leaf traits in a community of Mediterranean woody plants and investigated the relationships between leaf traits and other functional indices related to resource acquisition. We also evaluated the variation of functional leaf traits at the community level and its relationship to key environmental factors. Seventeen coexisting woody species were studied. Chemical leaf traits, in particular the content of micronutrients and non-essential elements, were more variable between species than morphological traits. The foliar isotopic content reflected functional aspects of the species, such as their water use efficiency or symbiotic interactions. The leaf mass per unit of area was highly related to several functional and environmental variables at the species and community level. Thus this foliar trait emerged as an important functional marker that indicated the species resources acquisition strategy, the species interaction with the environmental conditions and the functional dynamics of the community.

Subsequently, the thesis focuses on the two oak species that coexist in these forests, the sclerophyllous evergreen *Q. suber* and the winter deciduous *Q. canariensis*, and their relationships with the underlying soil. We first investigated the influence of the oak species on soil fertility. For that purpose we studied five components of plant-soil system namely living leaves, leaf fall, litter, superficial and sub-superficial soil. We detected a global gradient of nutrient enrichment common to plants and soils. The winter deciduous species *Q. canariensis* had enriched tissues and occupied the most fertile environments compared to the evergreen *Q. suber*. This suggested that both species occupied distinct biogeochemical niches in the ecosystem. A theoretical model that supported the existence of a tree-soil feedback process was tested using a causal modeling approach. Accordingly, the two oak species, through their different

nutrient return, would modify soil properties in a way that, in turn, could positively affect the presence of the species itself.

Additionally, we studied the effect of both *Quercus* species on the process of litter decomposition and investigated the synergistic dynamics associated with decomposition in mixed forests. We also analyzed the patterns of nutrient immobilization and release during decomposition. Species influenced decomposition through the quality of their litter and the different soil conditions they generate. The variation in the calcium content of the litter and soil explained a significant fraction of the observed differences in decomposition dynamics.

Next we studied the impact of the plant generated abiotic conditions in soil biological communities. We investigated the effect that season, vegetation cover type, including *Q. canariensis* and *Q. suber* cover, and soil depth had on the soil microbial carbon, nitrogen and phosphorus. There were seasonal changes in the microbial content of N and P, with higher values in spring and autumn. The microbial nutrient contents were higher beneath *Q. canariensis* and shrubbery cover than beneath *Q. suber* and in forest gaps. These differences were only significant in spring. Microbial C, N and P decreased significantly with depth in all cases studied.

Finally, we analyzed the diversity and structure of the ectomycorrhizal fungal community associated with the roots of *Q. canariensis* and *Q. suber* using molecular techniques for species identification. Three families dominated the fungal community: Telephoraceae, Russulaceae and Cortinariaceae. The distribution of symbiotic species was explained by the topsoil fertility and acidity conditions generated by the oak species. This indirect effect of host species on the ectomycorrhizal fungal community was related to a shift in the species composition and the phylogenetic diversity of the mycorrhizal community.

Índice

Capítulo 1.

Introducción general

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El conocimiento de las interacciones que se establecen entre las plantas y los suelos sobre los que se desarrollan es esencial para comprender el funcionamiento de los ecosistemas. Ya las primeras civilizaciones, como la egipcia o la romana, reconocieron que las propiedades de los suelos condicionaban el tipo de vegetación y la productividad agrícola (Hillel, 1991). Durante siglos el conocimiento popular distinguió entre especies forestales que degradaban las condiciones del suelo, como las coníferas, y especies que las mejoraban, como la mayoría de las especies frondosas de hoja caduca, lo que llevó a favorecer las formaciones de bosque mixto (Rennie, 1955; Urbieta et al., 2008). Sin embargo, el papel de la vegetación como elemento formador de los suelos no fue explícitamente reconocido hasta finales del siglo XIX por Dokuchaev (1879; en Attiwill y Adams, 1993). Posteriormente, Jenny (1941) consideró a la vegetación como uno de los cinco factores clave de la edafogénesis.

Tradicionalmente el suelo y la vegetación han sido estudiados independientemente por edafólogos y botánicos. Sin embargo, durante las últimas décadas se ha multiplicado el número de trabajos que abordan las interacciones planta-suelo desde un enfoque multidisciplinar, permitiendo así comprender el alcance de estas interacciones (Finzi et al., 1998a; van Breemen y Finzi, 1998; Copley, 2000; Wardle et al., 2004). La naturaleza dinámica e interactiva de las relaciones planta-suelo genera en ocasiones sistemas de retroalimentación que confieren estabilidad y resiliencia al sistema y desempeñan un papel fundamental en el control de las propiedades y procesos del ecosistema (Hobbie, 1992; Binkley y Giardina, 1998). Conocer los mecanismos de estas interacciones resulta crucial para tratar de prever la respuesta de los ecosistemas ante el cambio global.

INTERACCIONES PLANTA-SUELO EN SISTEMAS FORESTALES

El estudio científico de las relaciones entre planta y suelo en los sistemas forestales surgió hace más de 100 años, motivado principalmente por las repercusiones que las mismas tenían en la producción silvícola (Attiwill y Adams, 1993). Los primeros trabajos se centraron en estimar los balances de nutrientes en plantas y suelos, así como el retorno de nutrientes al suelo vía desfronde. Trabajos como el de Ebermayer (1876; en Attiwill y Adams, 1993) – *Comprehensive study of forest litter, with regard to the chemical statics of silviculture*- estudiaron la distribución de nutrientes, las tasas de transferencia de nutrientes entre la planta y el suelo, el modo en que el ciclo de nutrientes afectaba a las propiedades del suelo, las prácticas silvícolas que favorecían la productividad del ecosistema y sentaron las bases para el conocimiento de las interacciones planta-suelo. Posteriormente, el interés se centró en los procesos que controlan los ciclos de nutrientes. Se observó entonces que la disponibilidad de nutrientes estaba determinada por la competencia entre sumideros geoquímicos (suelo orgánico y mineral) y biológicos (plantas y microorganismos) y que estos últimos podían modificar activamente los procesos mediante, por ejemplo, la producción de exudados radicales (Attiwill y Adams, 1993).

En los sistemas forestales los árboles y el matorral son capaces de modular la disponibilidad (calidad, cantidad o distribución) de recursos necesarios para otros organismos tales como la luz, la temperatura, la humedad o los nutrientes, actuando como organismos ingenieros de ecosistemas cuyos efectos pueden además perdurar más allá de la muerte del individuo (Jones et al., 1994; Nilsson y Wardle, 2005; Mitchell et al., 2007). La capacidad de los árboles y el matorral para modificar las condiciones ambientales, así como la persistencia, intensidad y dirección de estos cambios, son características específicas de cada especie que interaccionan a su vez con otros factores como el sustrato o el clima (Eviner et al., 2006; Gómez-Aparicio y Canham, 2008). El efecto de las especies en el ecosistema, su huella, depende de múltiples rasgos, algunos de los cuales covarían mientras que otros varían independientemente del resto (Eviner y Chapin III, 2003). Los rasgos foliares están relacionados con la fenología y la tasa de crecimiento de la especie; rasgos como el área foliar específica y la concentración en nutrientes, compuestos secundarios, ligninas y ceras están muy relacionados con la huella específica (Cornelissen et al., 2006; Cornwell et al., 2008; Deyn et al., 2008). La estructura radical, la acumulación de reservas en la biomasa subterránea, la exudación de carbohidratos o las relaciones con organismos simbiontes como los hogos micorrícicos son también rasgos clave en las interacciones planta-suelo (Hobbie, 1992; Grayston y Campbell, 1995; Dijkstra y Smits, 2002; Deyn et al., 2008). El funcionamiento del ecosistema depende en parte de la combinación de rasgos funcionales (foliares y radicales) de su comunidad vegetal. El cambio o pérdida de diversidad de rasgos en una comunidad podría suponer una alteración en los procesos del ecosistema (Chapin III et al., 1998).

Los árboles y el matorral pueden influir en distintas características del ecosistema. Las especies forestales pueden condicionar los flujos de agua y las condiciones microclimáticas mediante distintos mecanismos (Prescott, 2002). Especies con una elevada área foliar específica interceptan un mayor porcentaje de precipitación, reduciendo la translocación de agua. Una mayor rugosidad de la corteza puede disminuir el flujo de escorrentía caulinar (Augusto et al., 2002). Diferencias en la fenología y fisiología de las especies influyen en los flujos de agua (Eviner y Chapin III, 2003). La longitud de las raíces, la capacidad de captación de agua por unidad de raíz, el tiempo en el que se desarrolla el sistema radical y la asociación con hongos simbiontes influyen en la capacidad de la planta para extraer agua del suelo y determinan la cantidad de agua disponible

(Gordon y Rice, 1993; Steudle, 2000; Querejeta et al., 2007). Las especies forestales pueden reducir la infiltración formando una capa hidrófoba en el suelo superficial a partir de sustancias como ceras, ácidos grasos, ácidos húmicos, resinas o aceites aromáticos procedentes de la hojarasca (Martínez-Zavala y Jordán-López, 2009). Además de alterar los flujos de agua, las especies arbóreas pueden modificar las condiciones microclimáticas bajo su copa modulando la entrada de luz a través de la fracción de cabida cubierta y el índice de área foliar; ambos parámetros son característicos de la especie (Canham et al., 1994). Del mismo modo las especies pueden alterar la temperatura del suelo y del aire, estrechamente relacionada con la entrada de luz (Hobbie et al., 2006). La cantidad y el color de la hojarasca acumulada en el suelo puede también influir en la temperatura del suelo (Facelli y Pickett, 1991).

Los suelos forestales son importantes depósitos de carbono y su capacidad para almacenar o liberar carbono está en parte determinada por las especies arbóreas y de matorral presentes (Deyn et al., 2008). Parámetros relacionados con la calidad de la hojarasca como la relación carbono:nutrientes, la concentración de lignina o la dureza de la hoja condicionan la tasa de descomposición de la biomasa (Cornwell et al., 2008; Meier y Bowman, 2008). La calidad de la hojarasca influye también en el límite de la descomposición, esto es, la fracción de hojarasca que alcanza un estado estable (con tasa de descomposición próxima a cero) y pasa a formar parte de la materia orgánica del suelo (Berg, 2000; Berg et al., 2003). La materia orgánica del suelo se deriva principalmente de las raíces muertas en descomposición ya que éstas poseen un mayor contenido en compuestos recalcitrantes y se encuentran protegidas entre los agregados del suelo (Gale et al., 2000). Especies de plantas que difieren en las distribución, composición y abundancia de sus raíces podrían generar una distinta acumulación de materia orgánica en el suelo. Las especies forestales, a través de la calidad de su hojarasca y raíces muertas controlan también en gran medida los procesos de mineralización e inmovilización de nitrógeno en los suelos (Gallardo y Merino, 1992; Lovett et al., 2004). Por ejemplo, los árboles influyen en la nitrificación mediante la producción de componentes que inhiben la microflora nitrificante así como alterando las condiciones de humedad y temperatura (Boerner y Koslowsky, 1989).

Las especies forestales pueden alterar los ciclos de nutrientes mediante distintos mecanismos; mecanismos químicos que generan cambios en la acidez del suelo y repercuten en la disponibilidad de los nutrientes; mecanismos físicos, como la lixiviación de elementos lábiles (K, Zn) desde las hojas; y mecanismos biológicos, como la distinta capacidad específica de absorción, alocación y concentración en el desfronde de elementos como el calcio, magnesio o azufre (Laskowski et al., 1995). Los árboles pueden aumentar la acidez del suelo mediante la absorción diferencial de cationes y aniones o el aporte de ácidos orgánicos exudados por las raíces o derivados de la hojarasca en descomposición. Por otro lado, las especies con elevados niveles de Ca y Mg en su desfronde o aquéllas que incrementan la capacidad de intercambio catiónico del suelo favorecen la capacidad de tamponar el pH del suelo (Finzi et al., 1998a; Reich et al., 2005; Gómez-Aparicio y Canham, 2008). La alteración de los niveles de acidez influye a su vez en la tasa de meteorización de los minerales (Quideau et al., 1996). La estructura de los suelos, su porosidad, densidad aparente y el estado de agregación de las partículas puede verse afectada por la características radicales (longitud y biomasa) de las especies forestales, la calidad y cantidad de los compuestos exudados por sus raíces y la asociación con organismos del suelo (Challinor, 1968).

Las alteraciones de las condiciones abióticas del suelo mediadas por las especies forestales repercuten en los organismos del suelo, cuyo entorno y recursos se ven condicionados por la cubierta vegetal. Los cambios en la acidez y fertilidad del suelo y las variaciones en la temperatura y humedad afectan a la abundancia y composición específica de la macro- y meso fauna (lombrices, ácaros, colémbolos, miriápodos, etc.), microfauna (nematodos, rotíferos y protozoos) y microflora (bacterias, hongos simbiontes, saprófitos y patógenos) del suelo (McAfee y Fortin, 1987; Myers et al., 2001; Reich et al., 2005; Mitchell et al., 2007; Negrete-Yankelevich et al., 2008). Las comunidades de organismos del suelo tienen un papel esencial en el ecosistema pues controlan la disponibilidad de nutrientes (Bardgett y Wardle, 2010). Las cadenas tróficas de organismos descomponedores en los suelos fértiles están dominadas por bacterias, nematodos, protozoos y lombrices que se asocian a elevadas tasas de descomposición y poca acumulación de carbono en el suelo. En cambio, los suelos ácidos y pobres en nutrientes acogen cadenas tróficas compuestas principalmente por hongos y artrópodos que dan lugar a una descomposición más lenta y a un mayor secuestro de carbono en el suelo (Wardle, 2002). La masa microbiana del suelo está relacionada con el proceso de mineralización de la materia orgánica (Jenkinson y Ladd, 1981). Las condiciones microclimáticas así como la calidad de los recursos proporcionados por las especies vegetales (hojarasca y raíces) y la distribución espacial y temporal de estos recursos son determinantes para las propiedades y el funcionamiento de la masa microbiana, y en último término, para la disponibilidad de nutrientes para la propia comunidad vegetal (Fisk y Fahey, 2001; Kara et al., 2008; Nielsen et al., 2009). La simbiosis con hongos micorrícicos es una relación ubicua e imprescindible para la nutrición de la mayoría de las especies vegetales (Smith y Read, 2008). En suelos deficitarios en nutrientes, como los forestales, esta relación se hace aún más necesaria. La composición de la comunidad de hongos micorrícicos está influida por las características del suelo (Brearley, 2006; Cavender-Bares et al., 2009). La especie huésped puede ejercer una selección directa sobre los organismos simbiontes con los que se relaciona (Tedersoo et al., 2008). A su vez, las especies vegetales podrían también alterar la composición de la comunidad simbionte mediante los cambios que inducen en las condiciones del suelo (Morris et al., 2008). Los cambios en la biota del suelo pueden provocar alteraciones en las propiedades físicas del mismo generando, por ejemplo, una mayor aireación y un aumento en la infiltración de agua que pueden modificar las tasas de descomposición y mineralización de la materia orgánica y la diversidad y composición de las comunidades vegetales (Wardle et al., 2004; Mitchell et al., 2007).

Los impactos de las especies forestales en la fertilidad del suelo y en los procesos del ecosistema han sido estudiados mayoritariamente en bosques monoespecíficos (Binkley y Valentine, 1991; Knops et al., 2002; Hobbie et al., 2006). Pero en los sistemas naturales las especies rara vez forman masas monoespecíficas, siendo más comunes las formaciones de masas mixtas donde coexisten diversas especies. Los trabajos realizados en bosques mixtos muestran que, incluso en estas situaciones, es posible detectar el efecto de cada especie en las propiedades del suelo creando un mosaico de fertilidad que refleja las características heterogéneas de la cubierta vegetal (Challinor, 1968; Boettcher y Kalisz, 1990; Finzi et al., 1998a). En los bosques mixtos las especies pueden interaccionar provocando efectos no aditivos que refuercen (sinérgicos) o anulen (antagonistas) el efecto individual de cada especie (Gartner y Cardon, 2004). La diversidad en las masas forestales genera heterogeneidad en los suelos y en otros parámetros ambientales lo que permite albergar una mayor diversidad de organismos y favorece la estabilidad y resiliencia del sistema. Los cambios en la composición de las masas forestales pueden tener, por tanto, una fuerte repercusión en las propiedades y procesos del ecosistema (Thompson et al., 2009).

LOS PROCESOS DE RETROALIMENTACIÓN

En los sistemas forestales, árboles y matorrales modifican las condiciones abióticas del suelo, lo que a su vez puede alterar la composición y estructura de las comunidades de organismos del suelo. Cuando los cambios en las condiciones abióticas y bióticas del suelo generados por las especies vegetales afectan al crecimiento de la propia planta o al establecimiento y supervivencia de sus propágulos se genera un efecto de retroalimentación que, a largo plazo, puede condicionar la composición de la comunidad y la distribución de las especies (Catovsky y Bazzaz, 2000; Ehrenfeld et al., 2005). Un efecto de retroalimentación puede ser negativo o positivo; así, por ejemplo, la acumulación de parásitos, patógenos o herbívoros en la rizosfera puede suponer una pérdida de biomasa y nutrientes de los tejidos de la propia planta, reduciendo su crecimiento y limitando el reclutamiento, lo que genera un efecto negativo. Lo contrario ocurre cuando, por ejemplo, las especies favorecen la asociación simbionte con especies de hongos micorrícicos que incrementan el acceso a nutrientes limitantes y generan un efecto positivo en la productividad de la planta (Wardle et al., 2004).

Los procesos de retroalimentación entre planta y suelo tienen gran importancia evolutiva. En algunos casos este efecto de retroalimentación se ha llegado a considerar como una extensión del fenotipo sujeto a selección natural ya que puede influir en su valor adaptativo (*fitness*) (van Breemen y Finzi, 1998). Desde un punto de vista evolutivo, los efectos de las especies vegetales en el suelo pueden ser resultado de distintos procesos. Binkley y Gardina (1998) distinguen tres niveles de interacciones; en un primer nivel se encuentran aquéllas en las que el efecto generado por la planta incrementa el valor adaptativo y genera ciclos de retroalimentación positivos. Los rasgos asociados a este efecto estarían sujetos a selección natural. En un segundo nivel aparecen los efectos que son consecuencia indirecta de rasgos seleccionados asociados a interacciones no relacionadas con el suelo, como por ejemplo, la acumulación de compuestos defensivos en el tejido foliar contra la herbivoría, que indirectamente influyen en la descomposición. En tercer lugar, quedarían los efectos que son resultado de interacciones ecológicas que no están sujetos a una selección evolutiva, como por ejemplo, el efecto de calidad de la hojarasca en la composición de la fauna del suelo. Estos dos últimos pueden ser o no ser favorables para la propia especie.

La principal dificultad que entraña el estudio de las interacciones planta-suelo y los procesos de retroalimentación radica en que la distribución de las especies a menudo responde a la variabilidad de otros factores (clima, topografía, suelo mineral), lo que impide aislar el efecto de la planta sobre las características del suelo (Van Breemen et al., 1997). En estos casos, existe una combinación compleja de los efectos de las especies sobre los procesos del ecosistema y de las condiciones ambientales (suelo, microclima) sobre las especies. A pesar de estas dificultades metodológicas, existen cada vez más evidencias, tanto observacionales como experimentales, que apoyan la hipótesis de que los árboles son capaces de modificar las condiciones del suelo y a su vez son afectados por estos cambios mediante ciclos de retroalimentación (Finzi et al., 1998a; Reich et al., 2005; Mitchell et al., 2007).

LAS INTERACCIONES PLANTA-SUELO EN EL BOSQUE MEDITERRÁNEO

Gran parte de los trabajos sobre las interacciones árbol-suelo han sido desarrollados en sistemas forestales templados y boreales. En este ámbito son numerosos los estudios sobre el efecto de las especies forestales en las condiciones de fertilidad de los suelos (Augusto et al., 2002; Dijkstra y Smits, 2002; Fujinuma et al., 2005), en los ciclos de C y N (Finzi et al., 1998b; Knops et al., 2002; Talbot y Finzi, 2008) en la composición de las comunidades de organismos del suelo (Mitchell et al., 2007; Negrete-Yankelevich et al., 2008; Malchair y Carnol, 2009) y sobre la interacción de dichas especies con otros factores, como las características del sustrato mineral (Van Breemen et al., 1997; Ste-Marie et al., 2007). En bosques boreales se ha prestado especial atención a las interacciones que establece el sotobosque (matorral, líquenes y musgos) cuya productividad en estos ecosistemas es comparable a la de las especies arbóreas (Nilsson y Wardle, 2005).

En los sistemas forestales mediterráneos, notablemente distintos a los anteriores y caracterizados por una acusada sequía estacional, se conocen menos estas interacciones, aunque existe un creciente interés sobre el tema como se desprende del elevado número de trabajos publicados en las últimas décadas. Las interacciones planta-suelo en ecosistemas forestales de clima mediterráneo han sido más ampliamente estudiadas en California, incluyendo aspectos relacionados con los ciclos biogeoquímicos y las interacciones con la biota del suelo (Quideau et al., 1996; Knops, 1997; Eviner et al., 2006; Querejeta et al., 2007; Morris et al., 2008). Menos numerosos son los estudios realizados en otros ámbitos de clima mediterráneo como Australia (Fonseca et al., 2000; Bennett et al., 2009; Meers et al., 2010), Sudáfrica (Richards et al., 1997; Ludwig et al., 2004) o Chile (Gutiérrez et al., 1993).

El papel de las interacciones planta-suelo en los ecosistemas de la cuenca mediterránea suscita actualmente un gran interés; por ejemplo se pueden ver los trabajos compilados en el número especial del *European Journal of Forest Research* sobre relaciones planta-suelo en bosques mediterráneos (Rubio et al., 2010) y los citados en la revisión de Gallardo et al. (2009) en el número monográfico de *Ecosistemas*. Los estudios desarrollados en países de la cuenca mediterránea tales como España, Italia o Portugal atañen diversos aspectos de las relaciones entre la vegetación y las condiciones bióticas y abióticas del suelo e investigan su interacción con factores como el sustrato o el clima. En particular se pueden mencionar los trabajos sobre el efecto de las especies vegetales en el proceso de descomposición de la hojarasca (Gallardo y Merino, 1992, 1993); el efecto de las encinas sobre el suelo y el pasto en las dehesas (citados en Marañón et al., 2009); las relaciones planta-suelo-microorganismo (García et al., 2002; Rutigliano et al., 2004; Goberna et al., 2007); y la influencia de las propiedades del suelo en el reclutamiento y supervivencia de distintas especies forestales mediterráneas (Gómez-Aparicio et al., 2005, 2008; Maltez-Mouro et al., 2009; Pérez-Ramos et al., 2010).

Los ecosistemas mediterráneos se caracterizan por varios atributos que determinan en gran medida la naturaleza de sus suelos y condicionan las interacciones ecológicas: el clima, la orografía, el fuego y el efecto milenario de la mano del hombre (Yaalon, 1997). La distribución estacional de la precipitación es el principal factor climático que condiciona los procesos de edafogénesis. El contraste de precipitación entre estaciones genera una limitación hídrica en la zona de enraizamiento que define un régimen de humedad xérico (Soil Survey Staff, 2010). La humedad que se produce en el invierno cuando la evapotranspiración potencial es mínima permite una moderada meteorización de los silicatos, facilita la disolución de las rocas carbonatadas y es particularmente efectiva para la lixiviación de los minerales arcillosos dispersados que se depositan en horizontes más profundos. La hidrología y los flujos laterales de agua provocan la iluviación de arcillas y carbonatos desde lo alto de las pendientes y su acumulación en las partes bajas, generando una diferenciación en los suelos de la catena. La estacionalidad tiene también importantes repercusiones en los procesos del ecosistema: el corto periodo en el que concurren una temperatura y humedad óptimas para la actividad microbiana (primavera y otoño) determina los pulsos de descomposición y mineralización de la materia orgánica y regula la disponibilidad de nutrientes. Las plantas y microorganismos del suelo que viven en estos ecosistemas deben adaptarse a la estacionalidad que marca la disponibilidad de recursos (Gallardo et al., 2009).

La cuenca mediterránea es, en general, montañosa y está dominada por paisajes con abruptas pendientes y valles relativamente estrechos, debido a que se sitúa en la zona de colisión entre dos placas. En esta región los suelos se han desarrollado mayoritariamente sobre calizas, dolomitas o esquistos poco metamorfizados cuya meteorización ha dado lugar a distintos tipos de suelos. La consecuencia directa de la dominancia de elevadas pendientes es la intensa erosión, que da lugar a suelos someros de meteorización limitada y pobres en nutrientes (Inceptisoles o Entisoles; Soil Survey Staff, 2010), excepto en los fondos de valle, donde se forman acúmulos de materiales erosionados. Los incendios, antes muy recurrentes y ahora más espaciados pero también más intensos, juegan también un papel fundamental en la dinámica de los ecosistemas mediterráneos, pues modelan la composición de las comunidades y mineralizan rápidamente la necromasa acumulada, produciendo pulsos de recursos disponibles (Shakesby et al., 1993; Hernández et al., 1997; Certini, 2005).

Quizás el rasgo clave de los ecosistemas mediterráneos es el factor antrópico que ha configurado los paisajes actuales y ejerce una gran influencia en las relaciones planta-suelo (Blondel y Aronson, 1999). Aunque la presencia de homínidos (*Homo antecessor*, *H. neanderthalensis*, *H. sapiens*) en esta región pueda ser muy antigua, el impacto humano y la deforestación comienza hace unos 8000 años con la colonización por los pueblos del Medio Oriente que trajeron la agricultura y la ganadería; el proceso de deforestación se intensificó con los griegos y romanos y continuó hasta la edad moderna. La pérdida de la protección natural que confiere la vegetación expone el suelo poco profundo a la desecación en el verano y a las lluvias torrenciales en invierno agravando los procesos de erosión (Naveh y Lieberman, 1984). Por otro lado, desde hace miles de años los bosques mediterráneos han sido sometidos a un fuerte manejo que ha buscado favorecer especies que proporcionaran recursos útiles, como la madera y la leña, o económicamente rentables, como el corcho, lo que ha resultado en un cambio en la composición de la vegetación y en la dinámica de los ecosistemas (Urbieta et al., 2008; Valladares et al., 2008).

PERSPECTIVAS ANTE EL CAMBIO GLOBAL

Asistimos actualmente a un proceso de cambio global en el que, a diferencia de épocas anteriores, los cambios biosféricos se están produciendo a una velocidad tal que dificulta la adaptación de los sistemas naturales (Ozcáriz et al., 2008). Las previsiones de cambio climático para las próximas décadas son particularmente severas en el caso de los ecosistemas mediterráneos (Bates et al., 2008). En el periodo entre 1950 y 2000 se registró una tendencia negativa de la precipitación en la cuenca mediterránea, que se proyecta en el futuro hasta alcanzar una disminución del 20% y un aumento significativo del número de días consecutivos sin precipitación. El incremento de las temperaturas y la disminución de la precipitación son en parte responsables de la pérdida de productividad forestal y del aumento de los incendios forestales en la región mediterránea. Los ecosistemas mediterráneos albergan una gran diversidad de especies vegetales y representan un punto crítico (*hotspot*) de biodiversidad a escala mundial (Médail y Quézel, 1999). El aumento de la aridez en estos ecosistemas, cuya vulnerabilidad aumenta con el cambio climático, tiene importantes repercusiones para la diversidad, pues conlleva la migración o la pérdida de especies (Thuiller et al., 2005). Una de las consecuencias negativas del cambio global es el aumento de especies invasoras. En los últimos años este fenómeno ha suscitado gran interés, pues se ha comprobado que los cambios en la composición de las comunidades debido a la invasión de especies exóticas puede llegar a modificar el funcionamiento del ecosistema y generar un efecto de retroalimentación que dé lugar a cambios irreversibles en la comunidad (Gómez-Aparicio y Canham, 2008; Marchante et al., 2008; Rodgers et al., 2008). En un estudio reciente Gritti et al. (2006) demostraron, mediante simulaciones, que numerosas islas mediterráneas podrían llegar a estar dominadas en un futuro por especies de plantas invasoras.

El conjunto de cambios en el uso del territorio constituye uno de los motores del cambio global y a su vez está determinado por el cambio climático, las mejoras tecnológicas y las presiones económicas (Watson et al., 2000). El cambio en los usos del suelo, bien sea la deforestación de bosques para usos agrícolas y ganaderos (pérdida de bosque) o la forestación de explotaciones agrarias abandonadas (ganancia), afecta directamente al intercambio gaseoso entre los ecosistemas terrestres y la atmósfera, al condicionar la capacidad de estos ecosistemas para fijar y retener carbono.

La contaminación atmosférica, resultado de la intensificación de las actividades humanas durante el último siglo, y el cambio climático están provocando importantes alteraciones biogeoquímicas en los ecosistemas (Schlesinger, 1997; IPCC, 2007). Estas alteraciones en los ciclos biogeoquímicos de los elementos pueden estar causando problemas ambientales como el decaimiento forestal y la pérdida de biodiversidad (Likens et al., 1996; Sala et al., 2001). Recientemente Sardans y Peñuelas (2004, 2005, 2007) han mostrado que en bosques mediterráneos, un incremento de la aridez limita la actividad enzimática y la disponibilidad de fósforo en el suelo, a la vez que modifica los patrones de acumulación de P y K en la vegetación. Estos cambios podrían generar un efecto de retroalimentación que alterase la dinámica biogeoquímica del ecosistema y con ella la composición de la comunidad.

Los motores (*drivers*) del cambio global afectan directamente al funcionamiento de los ecosistemas y es previsible que lleguen a provocar un cambio en la composición específica de nuestros bosques. En este contexto es esencial conocer el papel que desempeñan las diferentes especies en el funcionamiento de los ecosistemas forestales, entender cómo responderán dichos ecosistemas ante el cambio, y prever las consecuencias de una posible alteración de la composición de la comunidad forestal, para así poder desarrollar planes de manejo que aseguren la provisión de bienes y servicios ecosistémicos por el bosque. Entre estos servicios ecosistémicos a mantener, además de la producción directa de alimentos, madera, fibra y otros bienes, se cuentan los servicios de regulación de calidad del agua, clima, avenidas y residuos; los servicios culturales como el recreo y el disfrute estético y espiritual; y los servicios de soporte ecológico como la formación del suelo, la fotosíntesis y el reciclado de nutrientes (Thompson et al., 2009).

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Capítulo 2.

Estructura y objetivos de la tesis

Capítulo 2. **Estructura y objetivos de la tesis**

El objetivo principal de esta tesis doctoral es estudiar las interacciones ecológicas entre el suelo y las especies forestales de los bosques mediterráneos. Se estudian con particular hincapié dos especies de quercíneas que coexisten en estos bosques, la primera de hoja perenne y esclerófila y la segunda de hoja marcescente y no-esclerófila, y las relaciones que establecen con el suelo subyacente. Con esta investigación se pretende contribuir a una mayor comprensión de las interacciones planta-suelo en los ecosistemas forestales mediterráneos, aumentar la capacidad para una adecuada toma de decisiones acerca de su manejo sostenible y completar la base de conocimiento existente acerca de estas interacciones en otros ecosistemas.

Para la elaboración de esta tesis doctoral se realizaron trabajos descriptivos y experimentales en condiciones de campo. Los estudios se desarrollaron en el Parque Natural Los Alcornocales, situado en las provincias de Cádiz y Málaga. El Parque comprende un área aproximada de 170 000 ha y su vegetación está dominada por un bosque mixto de alcornoque (*Quercus suber* L.) y quejigo moruno (*Q. canariensis* Willd.).

El **capítulo 3** presenta una primera aproximación a las interacciones planta-suelo. Se estudia la heterogeneidad de rasgos foliares en la comunidad de plantas leñosas que componen estos bosques y se analiza la relación entre la distribución de estos rasgos y los gradientes de cobertura y fertilidad del suelo. Este estudio persigue contrastar la hipótesis de que los rasgos foliares, en particular el cociente de masa foliar por unidad de área (LMA, del inglés *leaf mass per area*), forman parte de la estrategia funcional de las especies vegetales, estando relacionados con la captación de nutrientes, la eficiencia en el uso del agua. Estos rasgos foliares pueden servir de predictores de procesos y propiedades del ecosistema, como la productividad o las tasas de descomposición.

Los **capítulos 4 -7** se centran en el estudio de las interacciones que el alcornoque y el quejigo moruno establecen con el suelo y de la huella que estas especies generan en el ecosistema. Los **capítulos 4 y 5** estudian los impactos que ambas especies de quercíneas producen en las condiciones abióticas del suelo. El **capítulo 4** investiga la influencia de las dos especies de quercíneas en las condiciones de fertilidad del suelo y evalúa el potencial efecto de reatroalimentación positiva que estas modificaciones pueden suponer para la propia especie. Para ello se estudian cinco componentes del sistema planta-suelo (hojas vivas, desfronde, hojarasca, suelo superficial y profundo) en tres rodales de bosque en los que se analizan la variabilidad y los patrones correlacionales de su composición química. Las hipótesis de trabajo son las siguientes: los elementos químicos ligados a componentes orgánicos, como por ejemplo el nitrógeno o fósforo, están sujetos a un mayor control biológico y por tanto su variabilidad en el ecosistema será menor; la transferencia de nutrientes entre los distintos componentes del ecosistema se verá reflejada en una estructura común de covariación de elementos; las dos especies de quercíneas, dados sus contrastados rasgos funcionales, ocuparán distintos nichos biogeoquímicos en el ecosistema, lo cual queda reflejado en una distinta composición química de sus tejidos y del suelo que ocupan; existe un efecto de retroalimentación positiva por el cual ambas especies, a través del distinto retorno de nutrientes vía hojarasca, modifican las condiciones del suelo de modo que favorecen la presencia de la propia especie.

El **capítulo 5** complementa al anterior ahondando en el proceso de descomposición y liberación de nutrientes a partir de la hojarasca. En particular, este capítulo busca conocer el mecanismo por el cual ambas especies de quercíneas pueden ejercer efectos directos, a través de la calidad de la hojarasca, e indirectos, a través de las condiciones ambientales, en el proceso de descomposición. Además, en este capítulo se investigan dos fenómenos asociados a la descomposición en bosques mixtos: el "efecto mezcla" que propone que la mezcla de hojarasca de distintas especies tiene una dinámica de descomposición diferente de cada especie por separado; y lo que se ha llamado “la ventaja de jugar en casa” (*home-field advantage*), un fenómeno según el cual la hojarasca tiende a descomponerse más rápidamente bajo la especie que la produce debido a una especialización de los organismos descomponedores. El capítulo se completa con un estudio de la química de la descomposición en el que se analizan los patrones de inmovilización y liberación de nutrientes durante el proceso de descomposición de la hojarasca. La principal hipótesis de trabajo, en concordancia con los resultados del capítulo anterior, es que el quejigo, al ser una especie caduca cuya hojarasca es más rica y el suelo subyacente es más fértil, tendrá una mayor tasa de descomposición y liberación de nutrientes que el alcornoque.

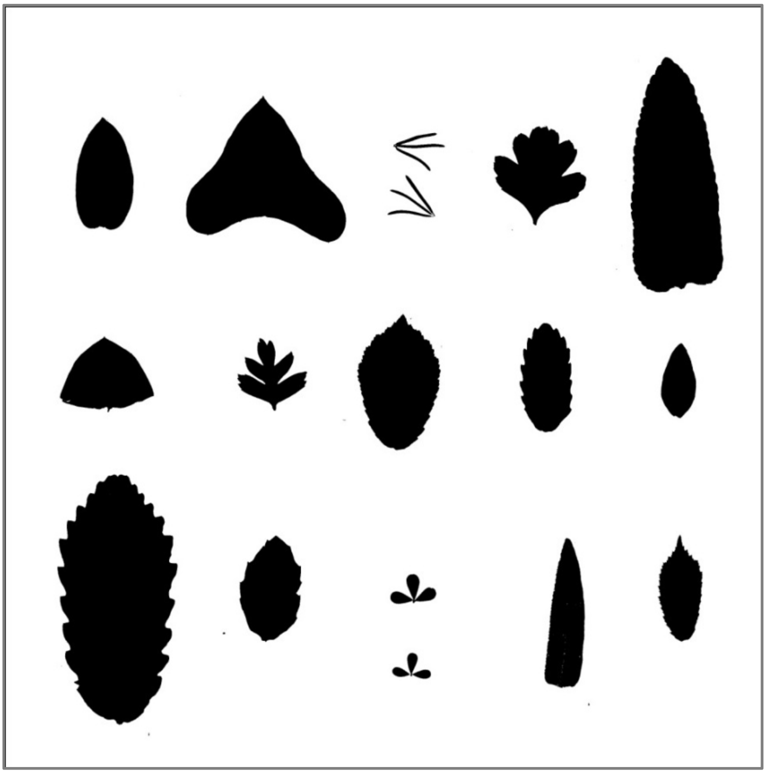
Los **capítulos 6 y 7** analizan las interacciones de estas especies de árboles con las comunidades biológicas del suelo. Las comunidades microbianas del suelo se ven afectadas por diversos factores que influyen en las condiciones físico-químicas del medio en el que estos organismos se desarrollan. El **capítulo 6** estudia el efecto que la estacionalidad, el tipo de cobertura vegetal, incluyendo la cobertura de alcornoque y de quejigo, y la profundidad del suelo tienen sobre el contenido en carbono, nitrógeno y fósforo microbiano de los suelos forestales mediterráneos. Las hipótesis planteadas son: la masa microbiana experimentará un mayor crecimiento e inmovilización de nutrientes durante la primavera mientras que decaerá en verano; el carbono, nitrógeno y fósforo microbiano será mayor en sitios más fértiles (bajo quejigo) que en sitios pobres en nutrientes y disminuirá con la profundidad del suelo.

El **capítulo 7** profundiza en el efecto que las dos especies de quercíneas tienen en un componente particular de la biología del suelo: los hongos ectomicorrícicos asociados a las raíces de ambas especies. Este trabajo evalúa la diversidad y estructura de la comunidad de hongos ectomicorrícicos empleando técnicas moleculares para la identificación de especies. Además se analizan las relaciones existentes entre la distribución de especies simbiontes y las características de la hojarasca y el suelo superficial. Se estudia en qué medida la distribución de especies simbiontes puede ser explicada por la identidad de la especie huésped -efecto directo- y por las condiciones del suelo superficial generadas por ambas quercíneas –efecto indirecto- utilizando modelos causales. La hipótesis es que ambas especies huésped, alcornoque y quejigo, a través de diferencias en la calidad de su desfronde, generan distintas condiciones en la hojarasca y el suelo superficial que afectan a la composición de la comunidad de hongos ectomicorrícicos.

La tesis concluye con un análisis conjunto de los resultados que permite una visión global de las interacciones planta-suelo en bosques mediterráneos y una evaluación de las implicaciones que estas interacciones tienen en el funcionamiento del ecosistema

Capítulo 3.

Heterogeneidad de rasgos foliares y su relación con el funcionamiento de las plantas y los ecosistemas mediterráneos



Este capítulo reproduce el siguiente manuscrito:

Domínguez M.T., Aponte, C., Pérez-Ramos, I.M., García, L.V., Villar, R., Marañón, T. *Leaf traits of Mediterranean woody plant species: is the leaf mass per area related to nutrient uptake and isotopic signatures?* Enviado a New Phytologist (Octubre 2010)

Capítulo 3. **Heterogeneidad de rasgos foliares y su relación con el funcionamiento de las plantas y los ecosistemas mediterráneos**

RESUMEN

Los rasgos foliares reflejan las estrategias de adaptación de las especies vegetales a las condiciones ambientales e influyen en los procesos del ecosistema. Los rasgos foliares químicos y morfológicos se encuentran interrelacionados, aunque el conocimiento de estas relaciones a escala de especie y de comunidad es aún escaso.

Hemos explorado el espectro de los rasgos foliares en una comunidad de plantas leñosas mediterráneas y hemos investigado las relaciones entre los rasgos foliares, en particular la masa por unidad de área (LMA), y otros índices funcionales relacionados con la adquisición de nutrientes, como los coeficientes de transferencia suelo-planta y las concentraciones isotópicas de carbono y nitrógeno. La variación de los rasgos foliares funcionales a nivel de comunidad y su relación con factores ambientales clave se estudió utilizando los rasgos foliares agregados

La masa por unidad de área se relacionó positivamente con la masa seca, el contenido en carbono, el ratio C:N y el contenido en ^{13}C (un “proxy”de la eficiencia en el uso del agua), mientras que la relación fue negativa con los coeficientes de transferencia suelo-planta de algunos nutrientes (N, Mg, S y Cu). A nivel de comunidad la variación en los valores agregados de LMA, C:N y $\delta\ ^{15}N$ se asoció a cambios en la densidad del dosel. La relación encontrada entre el LMA y múltiples variables a nivel de especie y de comunidad señala a esta característica foliar como un importante marcador funcional indicador de la estrategia de las especies en la adquisición de recursos, la interacción de las especies con las condiciones ambientales y la dinámica funcional de la comunidad. Este estudio aporta importante información sobre la relación entre los rasgos foliares, la estrategias funcionales y las condiciones ambientales a nivel de especie y comunidad y pone de manifiesto su relevancia como indicadores del funcionamiento del ecosistema.

Leaf traits of Mediterranean woody plant species: is the leaf mass per area related to nutrient uptake and isotopic signatures?

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Abstract

The variation in leaf traits among plant species influences their ecological success across different habitats and affects ecosystem processes. Morphological and chemical traits are intercorrelated and their complex relationships, at species and community scales, are not well understood.

We investigated the relationships between leaf mass per area (LMA) with the patterns of nutrients acquisition from soils and the isotopic signatures (C and N) for seventeen Mediterranean woody plant species. Then we explored the trends of leaf traits at community level, by weighting their values by the species relative abundance.

Across species we found a significant positive correlation between LMA and leaf dry mass, C content, C:N ratio and $\delta^{13}C$ *(a proxy for water use efficiency), while the correlation was negative between LMA and the transfer of some nutrients (N, Mg, S and Cu) from soil to leaves. At the community level, community weighted LMA, C:N and* $\delta^{15}N$ *were related to variation in canopy density. Since LMA was consistently allied to chemical and isotopic leaf traits, at the species and at the community level, it is strengthened as an important functional marker of species resource strategy, species response to environmental conditions and community functional dynamics.*

Key words: *community assembly, Mediterranean forest, nutrient stoichiometry, plant functional types, plant resource strategies, soil-plant nutrient transfer, specific leaf area, stable isotopes.*

INTRODUCTION

The evolutionary radiation of vascular plants has provided with a wide variety of leaf shapes and structures for a few common functions of intercepting light, fixing carbon and regulating water balance. That variation in leaf traits among plant species had a key role for their success in the colonization and persistence in very different climatic and habitat conditions worldwide (Wright et al., 2004; 2005; Cornwell and Ackerly, 2010). In addition, ecosystem processes such as carbon sequestration, nitrogen atmospheric deposition, nutrient return in senesced leaves and leaf litter decomposition and immobilization of chemical elements in soils are commonly driven by leaf traits (Cornwell et al., 2008; Deyn et al., 2008; Meier and Bowman, 2008).

Leaf traits are related among them by causal relationships that limit trait combination and impose a covariation with the result of trade-offs among functionally distinct traits (Cornelissen et al., 1999; Meziane and Shipley, 2001; Shipley et al., 2006). Two opposite functional strategies –conservative resource use strategy and resource acquisition strategy– can be distinguished from the range of trait variation that defines the leaf economics spectrum (Wright et al., 2004). Species with a conservative resource use strategy have high values of leaf mass per area (LMA), high-density tissues and long leaf life span (Coley, 1988; Reich et al., 1991; Reich et al., 1998). Consequently, their rates of leaf litter decomposition and mineralization are usually slow (Cornelissen et al., 1999). These morphological attributes allow to minimize nutrient demand and increase competitive abilities in nutrient-poor environments (Hobbie, 1992; Aerts, 1995). On the contrary, species with a resource acquisition strategy have leaves with low LMA, and high N concentration, two leaf traits commonly associated to rapid resource capture and higher relative growth rate (e.g., Poorter and Garnier, 1999; Wright and Westoby, 2001; Ruíz-Robleto and Villar, 2005). Through their nutrient-rich and more decomposable litter, these fast-growing species promote a rapid mineralization and a high rate of nutrient return, perpetuating the nutrient-rich environments were they are highly competitive (Cornwell et al., 2008).

Global scale analyses have shown that leaf mass per area (LMA), N and P concentration and photosynthetic capacity are intercorrelated forming a "leaf economics spectrum" and are fundamental traits driving plant functioning and ecosystem properties (Reich et al., 1999; Wright et al., 2004; Wright et al., 2005; Poorter et al., 2009). However, that intercorrelation at global scale may not be significant when analysed at a lower spatial scale (Wright et al., 2005). Independent analyses of leaf composition have identified the N and P main variation trend (the nucleic acid-protein set of elements according to Garten, 1978) but it differs from the trend for other elements such as Ca, K and Mg, having structural and enzymatic functions (Garten, 1978; Watanabe et al., 2007). There are few studies comparing leaf morphological traits, in particular LMA, with a more complete set of leaf nutrient traits (other than N and P) having contrasted variation trends (but see Fyllas et al., 2009). Moreover leaf nutrient concentration is a result of the plant nutrient acquisition process and the nutrient availability in the soil (Ordoñez et al., 2009; Elser et al., 2010). Correlating the transfer quotient (TQ, that is leaf:soil concentration ratio) of each particular mineral nutrient with the plant LMA we will gain better understanding of the relationships between the leaf traits and the nutrient acquisition capacity, than only using leaf concentration. As far as we know these relationships have not been explored before.

Isotopic signatures may also provide information about different strategies of resource acquisition. Leaf carbon isotope ratio ($\delta^{13}C$) has depleted values in plants because the discrimination against $^{13}CO_2$ during the carboxilation and diffusion processes. A linear relationship was found under controlled conditions between leaf $\delta^{13}C$ and the water use efficiency (WUE, carbon gained per water transpired) (Farquhar and Richards, 1984) leading to its widespread use to assess WUE in C3 plants (e.g., Farquhar et al., 1989; Monclus et al., 2006 but see Seibt et al., 2008 regarding independent variation of $\delta^{13}C$ and WUE). Comparative studies have frequently explored the relationships between LMA and $\delta^{13}C$ across plant species to detect functional trait syndromes (Lamont et al., 2002; Escudero et al., 2008).

Leaf nitrogen isotope ratio ($\delta^{15}N$) integrates distinct aspects of species N acquisition such as the source and the soil depth from where N is taken up, the association with mycorrhizal and symbiotic microorganisms, and the within-plant processes of N transport and assimilation (Högberg, 1997). The type of mycorrhizal interaction largely explains the differences in $\delta^{15}N$ among plants at a global scale (Craine et al., 2009), as well as among coexisting species at community scale (Hobbie et al., 2000). Ericoid and ectomycorrhizal stratregies, which usually are related with low leaf $\delta^{15}N$ values (Craine et al., 2009; Kranabetter and MacKenzie, 2010) are linked to resource-conservation attributes, such as low plant growth rates, low foliar nutrient concentrations and poor litter decomposability (Cornelissen et al., 2001; Meers et al., 2010). Negative $\delta^{15}N$ values in leaves may be indicative of a high dependence on mycorrhizal interactions for N uptake, which is increased under low soil N availability (Hobbie et al., 1999; Hobbie et al., 2000). There are few studies relating leaf morphological traits and $\delta^{15}N$ values (but see Rosado and Mattos, 2010).

Community-level leaf traits (weighted by the species relative abundance) may have strongly significant patterns along environmental gradients despite having weak or non-significant relations at individual species-level (Ackerly et al., 2002). Along a soil resource gradient, plants growing under resource-scarcity will have a different subset of functional traits from those in rich-resource habitats (Chapin III et al., 1993). Relatively few studies at the community level have investigated the covariation of leaf traits along those soil gradients (but see Gross et al., 2008; Fyllas et al., 2009; Ordoñez et al., 2009).

The general objective of this study was to explore the spectrum of leaf traits in a community of Mediterranean woody plants, and the relationships among morphological traits (mainly LMA) and the concentration of macro- and micronutrients, non-essential trace elements, C and N isotopes. We pursued three particular objectives: First, to analyze the relationships between LMA and nutrient acquisition patterns, using the TQs (leaf:soil concentration ratios). The hypothesis was that, among coexisting woody plant species, we would expect that plants with lower LMA (associated with resource acquisition strategy) will have higher transfer from soil to leaf, especially for those nutrients with a prevalent photosynthetic function. Second, to investigate the relationships between LMA and isotopic signatures of C and N. The hypothesis was that lower LMA species would have reduced water use efficiency and therefore lower leaf ^{13}C values. In addition those plants with low LMA (resource acquisition strategy) will have a lower dependence on mycorrhizal interactions for N-uptake, and thus they will have higher leaf ^{15}N values. Third, to explore the changes in leaf traits at community level along continuous environmental gradients and evaluate the consistency of relationships at the species and community levels. The hypothesis was that shifts in functional community traits are related to canopy density and nutrient and water availability. In particular, the community weighted LMA value is expected to increase with canopy density and lower levels of nutrient and water soil availability.

MATERIAL AND METHODS

Study area and species selection

The study area is located in the Aljibe Mountains, a protected mixed oak forest area in the southern of Spain. The climate is sub-humid Mediterranean-type with mild, wet winters alternating with hot, dry summers, and most rainfall (95%) occurring from October to May. The bedrock dominated by Oligo-Miocene sandstone originates acidic, nutrient-poor soils frequently interspersed with layers of marl sediments that yield soils richer in clay. The vegetation is dominated by the evergreen cork oak (*Q. suber* L.), mixed with the winter-deciduous Algerian oak (*Q. canariensis* Willd.), which is locally abundant in the valley bottoms (Urbieta et al., 2008). The study was conducted in a forest stand (La Sauceda; 36º 31’ 54’’N, 5º 34’ 29’’ W), located at 530-560 m of altitude on a NW facing slope. Mean annual temperature is 15.5º C and mean annual rainfall is 1470 mm. Tree density in the stand is relatively low, with 219 stems ha^{-1} and a basal area of 22 m^2 ha^{-1} (see more details about the forest site in Pérez-Ramos et al., 2008; Quilchano et al., 2008).

Seventeen woody plant species growing in the forest site were selected, including the dominant tree species (*Q. suber* and *Q. canariensis*) and the most abundant shrubs and vines in the understorey (Table 1). In the late spring of 2008, during the peak of vegetative growth, 32 plots (3 m x 3 m) were located within the forest site, covering the widest possible range of environmental conditions. In each plot, one individual (healthy and adult) of each abundant woody species was collected, up to five replicates per species, making a total of 85 individuals distributed among the 32 microsites. For each selected individual, we took at least two branches with young, fully expanded leaves, from those parts of the plant with the highest light exposition. The branches were placed in plastic bags and transported in a chilled, dark container to the laboratory where their bases were submerged in water at 15ºC during the night to fully rehydrate the leaves.

Each of these 32 plots was characterised, by analysing soil samples (25 cm depth) for physical and chemical properties. Soil texture was determined by the Bouyoucos hydrometer method; pH was measured in a 1:2.5 soil:water suspension; soil organic matter was determined by combustion at 450 ºC; total organic N was determined by Kjeldhal digestion. Available P was estimated by the Bray 1 method; Calcium, K and Mg were extracted with 1 M ammonium acetate and determined by atomic absorption spectrophotometry. Availability of micronutrients (Cu, Fe, Mn and Zn) and S was determined by EDTA solution and analysed by ICP-OES (inductively coupled plasma optical emission spectrophotometry; Thermo Jarrel Ash Corporation). See details on methods for soil analyses in Sparks (1996), and a data summary in Supporting Information, Table S1.

Table 1. Study species, family, life habit, leaf longevity and nutritional strategies. Arb-Shrub: arborescent shrub; ECT: ectomycorrhizal species; ARB: arbuscular-mycorrhizal species; ERI: ericodal-mycorrhizal species; N-FIX: nitrogen-fixing species; HPAR: hemiparasitic species.

Species	Code	Family	Life habit	Leaf longevity	Nutritional strategy
Crataegus monogyna Jacq	Cm	Rosaceae	Arb-Shrub	Winter deciduous	ECT
Cistus salvifolius L.	Cs	Cistaceae	Shrub	Evergreen	ECT
Calicotome villosa (Poir.) Link	Cv	Fabaceae	Shrub	Summer deciduous	N-FIX
Erica arborea L.	Ea	Ericaceae	Arb-Shrub	Evergreen	ERI
Erica scoparia L.	Es	Ericaceae	Shrub	Evergreen	ERI
Lonicera implexa Aiton	Li	Caprifoliaceae	Vine	Evergreen	ARB
Osyris alba L.	Oa	Santalaceae	Shrub	Evergreen	HPAR
Phillyrea latifolia L.	Phyl	Oleaceae	Arb-Shrub	Evergreen	ARB
Pistacia lentiscus L.	Plen	Anacardiaceae	Arb-Shrub	Evergreen	ARB
Phlomis purpurea L.	Pf	Labiatae	Shrub	Evergreen	ARB
Quercus canariensis Willd	Qc	Fagaceae	Tree	Winter deciduous	ECT
Quercus suber L.	Qs	Fagaceae	Tree	Evergreen	ECT
Rosa pouzinii Tratt.	Ros	Rosaceae	Shrub	Winter deciduous	ARB
Rubus ulmifolius Schott	Ru	Rosaceae	ShrubVine	Winter deciduous	ARB
Smilax aspera L.	Sa	Smilacaceae	Vine	Evergreen	ARB
Teucrium fruticans L.	Tf	Labiatae	Shrub	Evergreen	ARB
Teline linifolia (L.) Webb in Webb & Berthel.	Tl	Fabaceae	Shrub	Evergreen	N-FIX

Leaf trait measurements

Morphological traits

All measurements were conducted following the criteria defined by Cornelissen et al. (2003). Five rehydrated and fully expanded young leaves per individual were harvested and weighted to obtain the lamina and petiole fresh mass. Leaf thickness was measured with a micrometer (Electronic Digital Micrometer Comecta SA, Barcelona, Spain) (average of three measurements per leaf). For five species (*C. villosa*, *E. arborea*, *E. scoparia*, *O. alba* and *T. linifolia*), leaf thickness was not measured because it was lower than the micrometer resolution. Leaves were scanned and leaf area was determined by digital analysis of the images, using Image-Pro Plus 4.5 software (Media Cybernetic Inc. USA). Finally, the leaves were oven-dried at 70 ºC during 48 h and further weighed until the nearest 0.0001 g.

The Leaf Mass per Area (LMA, g m^{-2}) was calculated as the ratio between the dry mass of the lamina and its area. Likewise, the Leaf Dry Matter Content (LDMC, g g^{-1}) was calculated as the ratio between the dry and the fresh (saturated) weights of lamina.

Chemical traits

Additional leaves were taken from each individual (from the outer part of the crown) for chemical analysis. The lamina of leaves were separated, oven-dried (at 70 ºC during 48 h) and ground using a stainless-steel mill. Leaf carbon concentration was determined in an elemental analyzer (CHNS Eurovector EA-3000). Nitrogen was analyzed by Kjeldahl digestion. The rest of macronutrients (Ca, K, Mg, P, and S), micronutrients (Cu, Co, Fe, Mn, Ni and Zn) and non-essential trace elements (Ba, Cd, Pb) were extracted by wet oxidation with concentrated HNO_3 under pressure in a microwave digester. Macronutrients (except N) were analyzed by ICP-OES. Micronutrients and trace elements were analyzed by inductively coupled plasma mass spectroscopy (ICP-MS; Perkin Elmer, Sciex-Elan 5000). See details on methods in Jones and Case (1990). Several plant reference materials were analyzed to assess the quality of the analysis: NCS DC 73350 (white poplar leaves, China National Analysis Center for Iron and Steel) and BCR-62 (olive tree leaves, European Community Bureau of Reference). Carbon and N isotopic analysis of the samples were performed by using an EA-IRMS in continuous flow. The precision for both $\delta^{13}C$ and $\delta^{15}N$ analyses was about 0.2 per mil.

Nutrient acquisition by plants

We calculated soil-plant transfer quotients (TQ) for each nutrient, as the ratio between the foliar and the available soil concentrations, to assess species-specific strategies of nutrient acquisition. For that purpose, we used the data of soil availability for the same elements determined in the leaves and taken in the same 32 plots (see above).

Community-level leaf traits along environmental gradients

To study the variation in leaf functional traits at community-level we calculated community-aggregated leaf traits. For that purpose, we selected 60 plots within the forest site that included most of the 32 plots where plants species were collected for leaf trait analyses (see above). These plots had been characterized in a parallel study focused on seedling growth (Pérez-Ramos et al., 2010). The relative abundance of the woody plant species was visually estimated as cover percentage in each plot. To scale up from species to community level, Community Weighted Means (CWM, sensu Violle et al., 2007) were calculated for the leaf traits showing the lowest intra-specific variability - LMA, LDMC, C concentration, C:N ratio, $\delta^{13}C$ and $\delta^{15}N$ - (see Supporting Information). The CWM values integrate the information on community structure (local abundances of species) and on the functional identity of species. We analyzed the changes in CWMs in relation to some key environmental factors, namely canopy density, water and nutrient availability (see Supporting Information, Table S2).

Data analysis

Coefficients of variation (CV) were calculated to assess the traits variability at the species and the community level. Principal component analysis (PCA) of 20 leaf traits (morphological, chemical, and isotopic signatures) and 85 plant individuals were performed to explore the multivariate patterns. Leaf thickness was excluded from the data set for the multivariate analysis, since measurements could not be obtained for some species.

Bivariate relationships between species leaf traits, averaged and log-transformed, were explored with Standardized Major Axis slopes (SMAs, also known as reduced major axis slopes) which have been recommended for allometric studies (Warton, 2006). SMA slopes are fitted by minimizing sums of squares in X and Y dimensions simultaneously and they give the proportional relationship between variables, that is how one variable scales against another. SMA regressions were performed by SMAR-software ver. 2.0 (Falster et al., 2006). SMA fitting was also applied to explore the relationships between LMA and nutrient transfer quotients (TQ). Since intraspecific variability of the transfer quotients was higher than that of leaf traits, measurement error variances were considered during the SMA fitting, as suggested by Warton et al. (2006).

Relationships among community weighted means, as well as their variations along environmental gradients were assessed by Pearson correlation analyses. We controlled at the 5% level the increment of type I error derived from multiple testing using a ‘false discovery rate’ (FDR) procedure (Hochberg, 2000) as suggested by García (2003).

RESULTS

Leaf trait variability

Across the studied species, there was a strong variability in the leaf traits. Leaf area was the most variable morphological trait varying across three orders of magnitude. The highest LMA values (> 140 g m^{-2}) were shown by *Q. suber* and *T. linifolia* while the summer deciduous *C. villosa* and the winter deciduous *C. monogyna*, *R. pouzinii* and *R. ulmifolius* had the lowest LMA values (< 74 g m^{-2}) (see Supporting Information, Table S3).

In general, micronutrients and non-essential elements had a greater variability than macronutrients (Supporting Information, Tables S4 and S5). Carbon concentrations were relatively stable among species (CV < 7 %), while some macronutrients, such as K, Ca, Mg and P showed greater interspecific differences. Isotopic signatures were more variable for ^{15}N than for ^{13}C (Supporting Information, Table S6). Nitrogen isotopic composition was associated with the type of symbiotic/parasitic relationships that are known to be established by the studied species (Table 1): N-fixing species (legumes) and the hemiparasitic species (*O. alba*) had positive values of δ^{15}N while the rest of species with different mycorrhizal associations showed negative δ^{15}N values (Supporting Information, Fig. S1).

Links among leaf traits

In the multivariate analysis of leaf traits (by PCA) the first axis (26.6 % of variance) clearly separated *O. alba* from the rest of species, due to their high concentration of P, Ca, Mg and S associated to the parasitizing capture of nutrients. A second PCA was carried out (for 16 species, excluding *O. alba*) to gain insight in the general leaf trait patterns. Then, the first PCA axis (explaining a 26.2 % of variance) was positively related to LMA, LDMC, C, C:N ratio and δ^{13}C, and negatively related to the concentrations of all macronutrients (with the exception of P) and micronutrients (Fig. 1, Supporting Information, Table S7). The second PCA axis was most strongly correlated with the N-related variables, such as N:P and δ^{15}N, as well as the Mn and Ni concentrations. The coordinates of the species in the space defined by first and second PCA-axes reflected the different foliar strategies of the species (Fig. 1). Winter deciduous (such as *C. monogyna* and *R. ulmifolius*) and legume (*C. villosa* and *T. linifolia*) species were located towards the nutrient-rich (negative) end of the axis 1 in opposition to species with higher δ^{13}C and LMA such as *Q. suber*, *P. lentiscus* and *P. latifolia,* that were in the positive end. Symbiotically nitrogen fixing (legume) species were clearly separated on the negative end of axis 2 (Fig. 1).

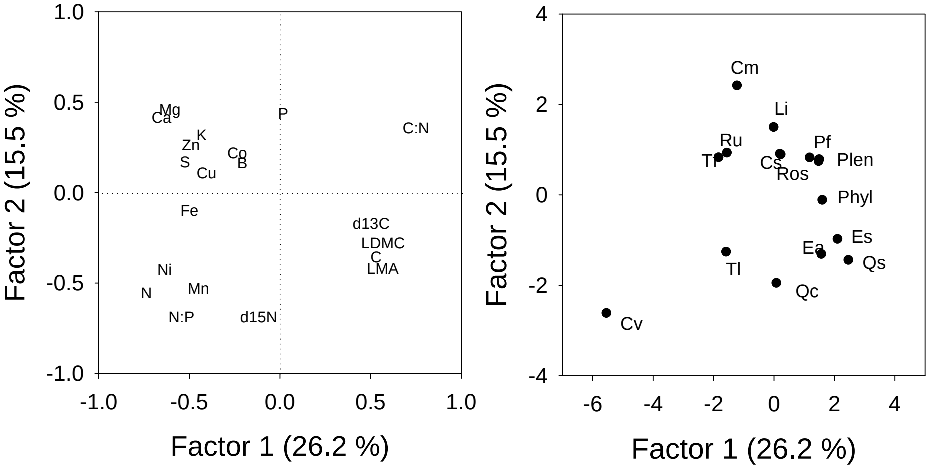

Figure 1. Ordination of leaf traits (left) and woody plant species (right) in the space defined by the first and second PCA axes. See species codes in Table 1 and variable abbreviations in table S5.

The bivariate relationships confirmed the general patterns revealed by the PCA. Leaf mass area (LMA) was positively related to N and P content on an area basis (Fig. 2 a, b), but not on a mass basis. For both elements, SMA slopes were not significantly different from one (Supporting Information, Table S8), indicating an isometric relationship (1:1) between LMA and the N and P concentration. Only for two elements (Mg and Pb) we found a negative relationship with LMA (Mg, r^2= 0.35, p< 0.01, slope: -1.7; Pb, r^2= 0.28, p< 0.03, slope: -2.6), besides for Ca and Cu there was a marginally significant negative relationship with LMA (*P*<0.10).

Leaf mass per area showed a negative relationship with Mg and Pb (Mg, r^2= 0.35, *P*= 0.01, slope: -1.7; Pb, r^2= 0.28, *P*= 0.03, slope: -2.6) and a marginally significant negative relationship with Ca and Cu (p< 0.10). For these nutrients, LMA scaled with an SMA slope steeper than 1, thus the ratio of nutrient concentration to LMA increased towards the high nutrient-low LMA end of the trait relationship.

With regards to isotopic signatures, LMA was positively and significantly related to δ^{13}C, with a SMA slope of 4.2 (Fig. 2d), suggesting that high LMA species had higher δ^{13}C to LMA ratio, thus were exponentially more efficient in their use of water than low LMA species. However, no significant relationship between δ^{15}N and LMA or any other morphological leaf trait was observed (Fig. 2c). A summary of the bivariate relationships between LMA and the rest of leaf traits can be found in the Supporting Information (Table S8).

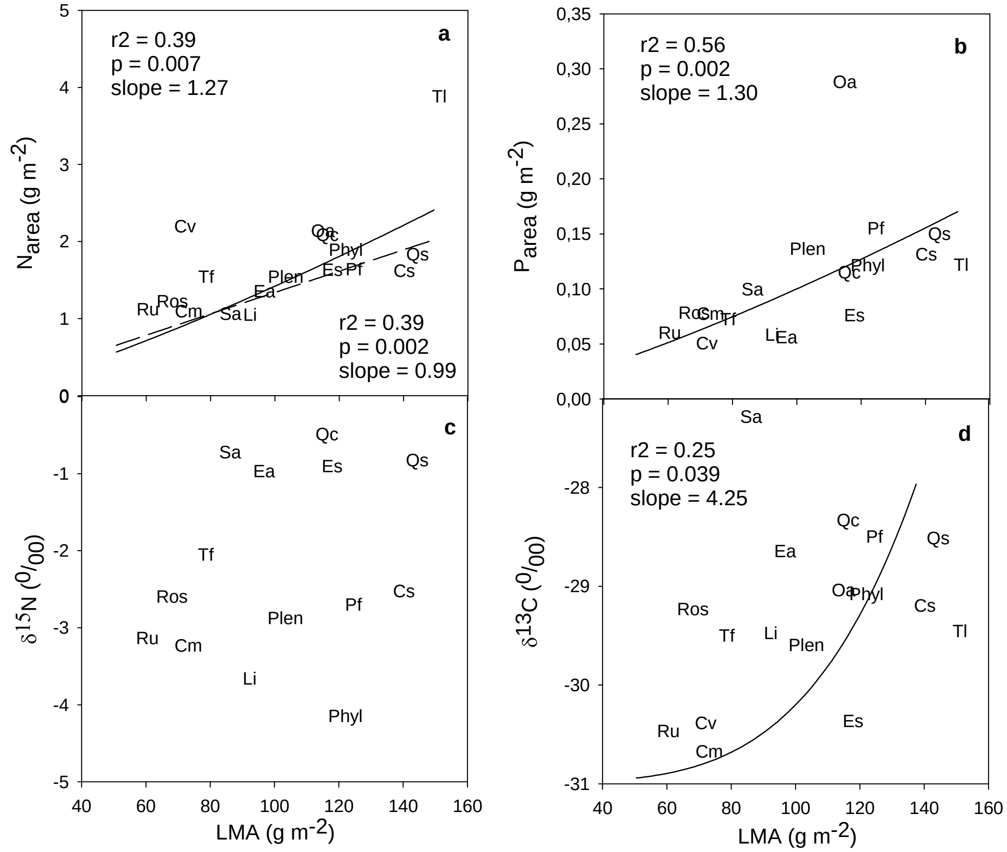

Figure 2. Bivariate relationships between leaf mass per area (LMA) and other leaf traits, analyzed by standardized major axis (SMA) regressions. See Table 1 for species codes. When correlations were significant, r^2 coefficients, *p*-values and slope values were indicated. The LMA-N_{area} relationship was calculated for all the species (upper solid line), and also excluding N-fixing species – *C. villosa* and *T. linifolia* – (lower dashed line). The LMA-P_{area} relationship was calculated excluding the hemi-parasitic *O. alba* (Oa) from the data set. See Supplementary Information (Table S8) for a complete report of the results of the bivariate correlations.

LMA and nutrient acquisition

There was a wide variation in the transfer quotients (TQ) from soil to plant depending on the nutrient, ranging from average (all species) values of 1060 (for N) and 469 (for P) down to 0.54 (for Fe) and 0.11 (for Ni) (Supporting Information, Table S9). In general, micronutrients showed higher levels of TQ variability (e.g., CV of 105% for Mn) than macronutrients (e.g., CV of 32% for K).

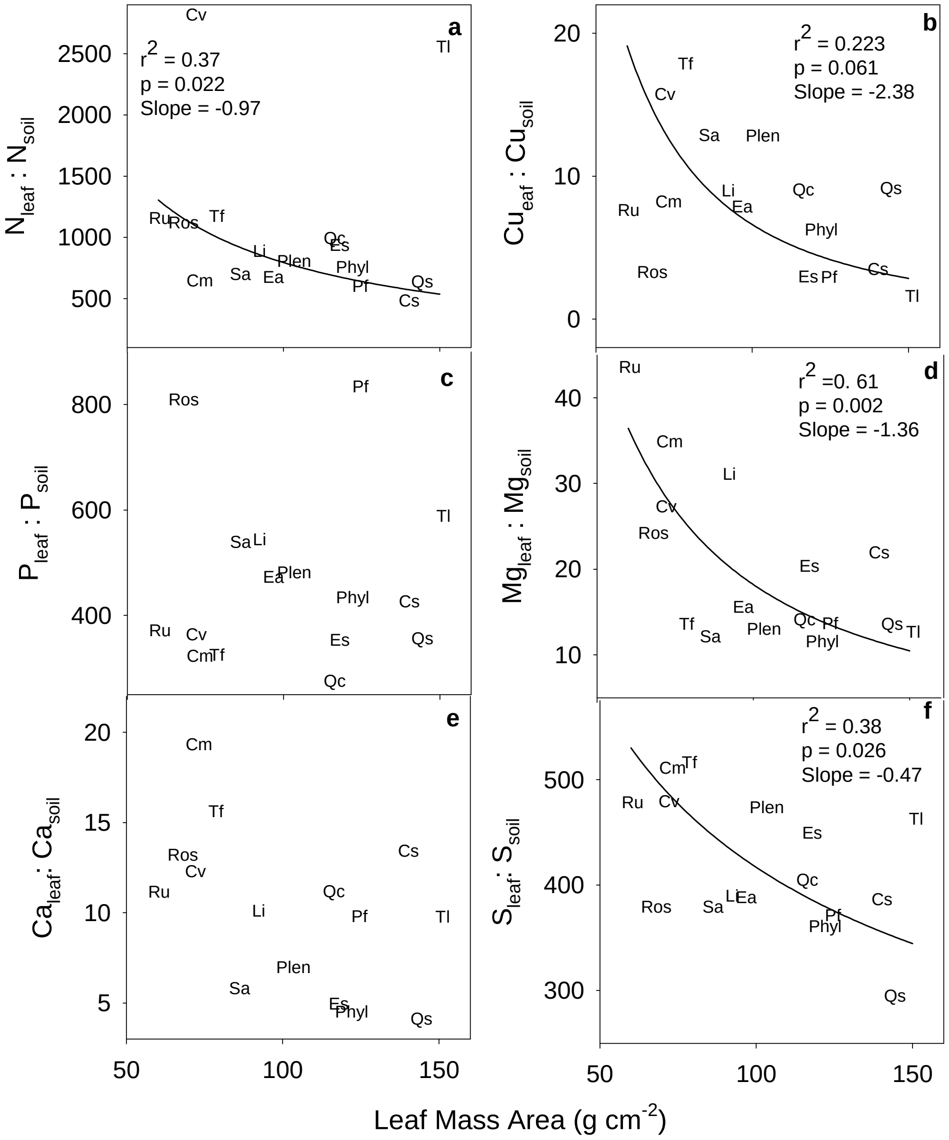

Figure 3. Relationships between transfer quotients (TQ, leaf : soil concentration ratios) and LMA (leaf mass per area), analyzed by SMA. See Table 1 for species codes. The LMA-N relationship was calculated excluding N-fixing species – *C. villosa* and *T. linifolia* –. When relations were significant, r^2 coefficients, *p*-values and slope values were indicated. See Supplementary Information (Table S10) for a complete report of the results of the bivariate correlations.

There were significant and negative relationships between LMA and TQ for some elements, namely N, Cu, Mg and S suggesting a higher nutrient acquisition from soil by the lower-LMA species (Fig. 3, Supporting Information, Table S10).

LMA showed an isometric relationships (SMA slope equal to one) with the TQ of N (after excluding the N-fixing species). SMA slopes differed from one ($p< 0.05$) for S and Cu suggesting that for these elements the ratio between nutrient acquisition capacity (TQ) and LMA varied across the LMA range (Fig. 3). The transfer of the rest macronutrients, such as P and Ca, was not related to LMA (Fig. 3c, e), although for Ca was marginally significant ($p= 0.07$, slope= -1.78).

Community weighted traits along environmental gradients

There was a large variation in the plant cover across the sampled communities and, consequently, in the canopy density (represented by the total Leaf Area Index, LAI) and the level of light intercepted by the canopy (estimated as the Global Site Factor, GSF), associated to a differential distribution of woody plant species (Supporting Information, Fig. S2). The gradient ranged from open shrublands with LAI 0-0.5 m^2 m^{-2}, and dominated by small-leaved species such as *C. villosa* (72% of cover) and *E. arborea* (17%), up to dense forests (LAI 2-3 m^2 m^{-2}), with overstorey of *Q. suber* (14-23%) and *Q. canariensis* (11-17%), and a dense understorey of arborescent *P. lentiscus* (25-35%), *P. latifolia* (8-16%) and *E. arborea* (5-17 %).

When analyzing the bivariate relationships between the community weighted traits considered in this study, we found significant relationships between LMA_{CWM} and variables such as $\delta^{15}N_{CWM}$ and $C:N_{CWM}$ ratio that were not observed at the species level (Fig. 4a, c). On the other hand, the positive relationship between LMA and $\delta^{13}C$ was maintained both at the species and at the community level (Fig. 4b, Supporting Information Table S11).

Several community leaf traits were significantly related to LAI and light availability at ground level (GSF) (Supporting Information, Table S12) both variables related to canopy density. Thus, $\delta^{15}N_{CWM}$ linearly decreased with LAI whereas LMA_{CWM} and leaf $C:N_{CWM}$ increased with canopy density (Fig. 5). Only few leaf tratits (e.g. $C_{CWM,}$ $\delta^{13}C$) showed significant or marginally significant relationships with soil moisture and soil nutrient concentration. Soil moisture was only significantly related to C_{CWM} ($r = -0.37$) and $\delta^{13}C_{CWM}$ ($r = 0.35$), whereas soil nutrients showed few marginally significant relationships.

DISCUSSION

Spectrum of leaf traits in a Mediterranean woody plant community

The studied forest community showed high interespecific variability in leaf traits for a set of coexisting woody species. Variability (CV) was greater for chemical than for morphological traits, with the exception of the leaf size. The low variability of traits such as LMA or C concentration indicates that they are

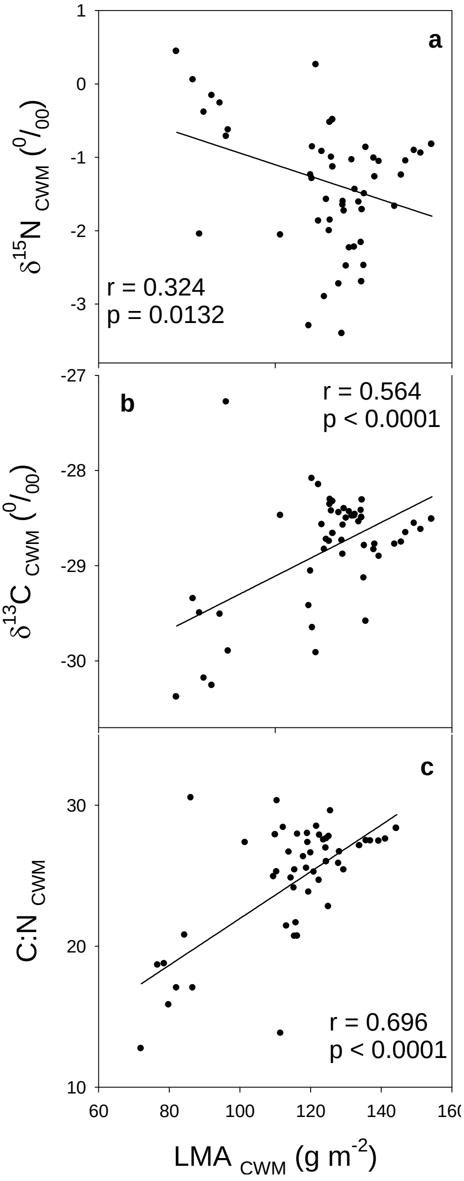

Figure 4. Bivariate relationships between community-weighted leaf mass per area (LMA) and isotopic discrimination of ^{15}N (a) and ^{13}C (b), and the leaf C:N ratio. CWM: Community Weighted Mean.

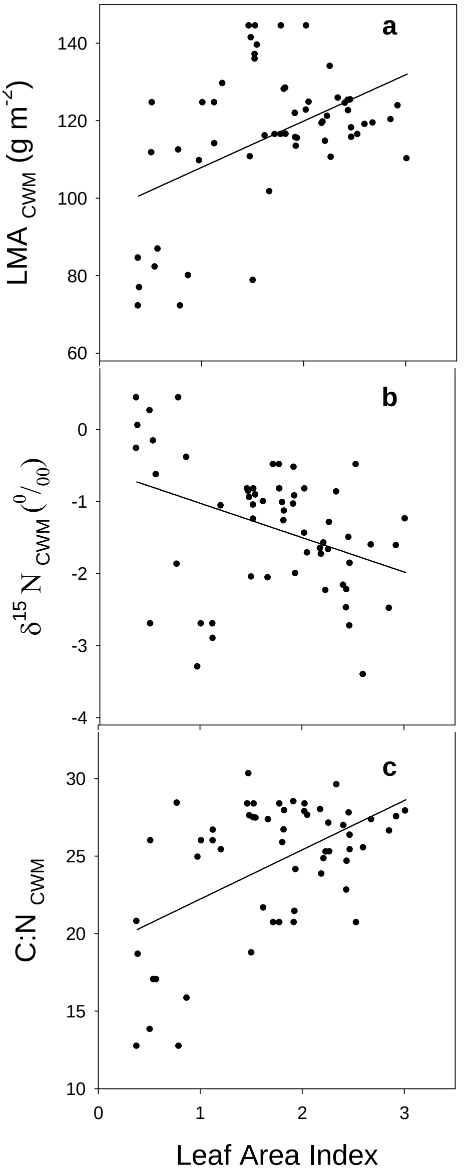

Figure 5. Relationship between canopy density (estimated as leaf area index) and community-weighted leaf mass per area (LMA, a), isotopic discrimination of ^{15}N (b), and leaf C:N ratio (c). CWM: Community weighted mean.

subjected to a strong homeostatic regulation and suggests that these traits play a key role in plant functioning (Wright et al., 2005; Poorter et al., 2009).

The leaf concentrations of macronutrients were less variable than those of micronutrients and non-essential elements, as was found at global and regional scales (Wood et al., 2006; Watanabe et al., 2007). The nutrient variability depends on its degree of biological control, chemical bonding properties and sources. Thus S and N, which form covalent bonds with organic molecules, have low variability while Ca, Mg and K, which are originated from mineral weathering, are more variable (Ladanai et al., 2010).

Isotopic signatures were more variable for $\delta^{15}N$ than for $\delta^{13}C$. Carbon discrimination is driven by stomatal conductance and internal diffusion, which are two mechanisms under a strong homeostatic control (Lamont et al., 2002). In contrast, leaf $\delta^{15}N$ can be influenced by several factors such as the source (soil, precipitation, N_2 fixation), the depth in soil from which N is taken up, the form of soil-N used (organic N, NH_4^+ NO_3^-), the mycorrhizal symbioses, the fractionation processes during and after N uptake by plants, and the interactions between these factors and plant phenology (see review by Högberg 1997). We have found here that nitrogen isotopic signature discriminated among functional groups of symbiotic interactions and thus emerges as a possible marker for mycorrhizal habit (Högberg, 1990). N-fixing (legumes) and hemiparasitic species (*O. alba*) had positive values of $\delta^{15}N$ while the rest of species with different mycorrhizal associations showed negative $\delta^{15}N$ values. Interestingly, the lowest $\delta^{15}N$ values were shown by arbuscular plants, in contrast to other studies in different ecosystems which found that arbuscular plants had the highest $\delta^{15}N$ values among mycorrhizal types (Högberg, 1990; Michelsen et al., 1998). Further studies on $\delta^{15}N$ partition are needed to comprehend the observed pattern.

The results of the multivariate analysis showed a relation between plant functional groups and leaf traits, reflecting a trade-off between resource acquisition and resource conservation strategies (Wright et al., 2004). Thus, resource-conservative species had high LMA, LDMC, C, C:N ratio (sclerophyllous type) and high $\delta^{13}C$, as opposed to those resource-acquisition species with low water use efficiency (low $\delta^{13}C$) and nutrient-rich and thin leaves (Poorter and Garnier, 1999; Wright and Westoby, 2001).

A second trait-based gradient distinguished a small group of species with a particular N economy (legumes), associated with N-fixing bacteria, having higher N concentration and $\delta^{15}N$, despite a variable leaf morphology. A singular case was the shrub species *Osyris alba* wich had leaf morphological traits within the values of the community, but was very distinct in terms of nutrient composition, due to its parasitic nature.

LMA and plant nutrient economy

In general, in our study LMA was not related to nutrient concentration on a mass basis. This contrast with the consistently negative relationship of LMA and P concentration found for global datasets (Wright et al., 2004; Wright et al., 2005; Ordoñez et al., 2009; Liu et al., 2010). This might indicate that global patterns may not hold for some regional scales as found by Wright et al. (2005).

Regarding the scaling of the relationships, LMA was positively and isometrically related to N_{area} and P_{area}, suggesting a constant increase of N and P per unit of leaf mass area gained. In contrast, LMA scaled steeper with $\delta^{13}C$ (SMA slope> 1) and some other nutrients (Mg, Ca, Pb, Cu) (SMA slope< -1), thus the ratio between water use efficiency or nutrient content and LMA varied across species LMA range.

When we analysed the nutrient acquisition capacity through the transfer quotients, we found significant negative relationship between species LMA and their capacity to acquire nutrients (for N, S, Mg and Cu). Plants with lower LMA usually have higher transpiration rates that could be related to a higher acquisition capacity of soil nutrients and a faster growth (Poorter and Garnier, 1999; Wright and Westoby, 2001) explaining the trend found for some elements. However, P transfer was not related with LMA, despite its importance as limiting nutrient in this system (García et al., 2006), thus its acquisition was probably more determined by root traits and mycorrhizal association (Bolan, 1991). More research is needed to fully understand the mechanisms relating leaf traits and nutrient acquisition processes.

Are LMA relationships consistent at the community level?

The positive relationship between LMA and $\delta^{13}C$ was consistent at both the species and the community level, supporting the strong association of leaf morphology (LMA) with plant water use efficiency reported by previous studies (e. g., Cunningham et al., 1999; de Bello et al., 2009). However, the relationships between LMA with $\delta^{15}N$ and C:N ratio found at community level were not significant at the species level. That is, significant relations between leaf traits at community level might not be maintained at species level (Ackerly et al., 2002).

The functional matrix of the community was correlated with the canopy density and the light availability at ground level. The canopy density was negatively related to the LMA_{CWM}, $C{:}N_{CWM}$ and positively with $\delta^{15}N_{CWM}$. On one side of the gradient, woody species with arbuscular and ericoid mycorrhizae (low foliar $\delta^{15}N$) and sclerophyllous leaves (higher values of LMA and C:N) tend to form denser forests and shadier understorey, and to exclude juvenile light-demanders (Canham et al., 1994; Sack et al., 2003). On the other side, open microsites are dominated by a legume shrub (*C. villosa*) having a particular summer-deciduous leaf habit (with lower LMA) and symbiotic N-fixation ability (so higher $\delta^{15}N$ and lower C:N), therefore contributing to the observed peculiar community patterns. This successful combination of traits contrasts with other semi-arid shrub communities dominated by non-legumes with evergreen leaves.

In spite of the widely recognized importance of soil water and nutrients as main limiting resources for plants in Mediterranean conditions, we did not find any significant relation between them and the LMA_{CWM}, at the forest stand-scale explored.

Leaf traits and ecosystem properties

Community species composition is the result of multiple hierarchical abiotic and biotic filters that select for species with a set of adequate traits to persist under particular local conditions (Keddy, 1992; Diaz et al., 1999). These plants traits are at the same time cause and result of the ecosystem functioning (Lavorel and Garnier, 2002). For example, plant traits have been amply used as strong predictors of interspecific variation in plant productivity or litter decomposition (Knops et al., 2002; Cornwell et al., 2008). To scale up from organ to ecosystem and generate reliable terrestrial ecosystem models it is necessary to select a series of soft traits, i.e. traits easily measured that have, at the same time, good predictive power of important ecosystem processes (Hodgson et al., 1999). In this sense, traits related to resource acquisition strategy are important because they represent species responses to resource availability and also influence biogeochemical cycling (Schimel et al., 1995; Lavorel and Garnier, 2002; Chapin III, 2003; Cornelissen et al., 2006). Among those leaf traits, LMA, LDMC, and N have been selected as functional markers influencing ecosystem productivity, litter decomposition and soil C and N content (Wright et al., 2005). In this study LMA has shown significant relations with N and P (on area basis) at species level, and with C:N and isotopic signatures at community level, having consequences for leaf litter decomposition and mineralization (De Angelis et al., 2000; Pérez-Harguindeguy et al., 2000) .

Species, through their leaf traits, modify ecosystem abiotic and biotic properties, such as incident light, soil fertility and soil decomposer communities, what in turn may have consequences on plant community (Mitchell et al., 2007; Aponte et al., 2010a,b; Pérez-Ramos et al., 2010). Understanding the mechanisms behind the interactions existing between traits and ecosystem functioning is essential to gain insight into community assembly rules and to foresee the consequences of community and traits diversity loss on ecosystem properties resulted from the ongoing global change (Wright et al., 2005).

CONCLUSIONS

The main conclusions drawn from this study were as follows:

(i) Leaf isotopic signatures reflect species functional aspects such as water use efficiency ($\delta^{13}C$) and symbiotic/parasitic interactions ($\delta^{15}N$), and are related to other functional traits (LMA, N and C:N) at the community level. Thus, they emerge as potential indicators of shifts in community functional composition.

(ii) Within the community range of leaf morphological traits some species can be very distinct in terms of nutrient composition because of their extra uptake by root-parasitising.

(iii) Canopy density (which conditions light availability) emerges as the factor most closely related to LMA_{CWM}, $C{:}N_{CWM}$ and $\delta^{15}N_{CWM}$ while soil water content or nutrient concentrations do not have a significant correlation with those traits.

(iv) LMA is significantly related to important variables such as LDMC, leaf N and P, and transfer of nutrients from soils to leaves, at species-level, and with C:N, $\delta^{13}C$ and $\delta^{15}N$ at community-level. Therefore, it is consolidated as an important functional marker indicative of species resource strategy, response to environmental conditions (light interception) and community functional dynamics.

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SUPPORTING INFORMATION

Figure S1. Leaf $\delta^{15}N$ values of the different functional groups of symbiotic/parasitic interactions. ECT: ectomycorrhizal species; ARB: arbuscular mycorrhizal species; ERI: ericoid mycorrhizal species; N-FIX: Nitrogen-fixing species; HPAR: hemiparisitic species.

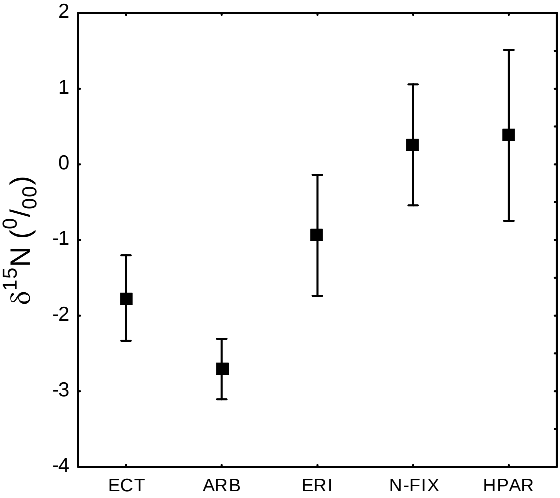

Figure S2. Relative abundance (% cover) of main woody species in the community across different classes of canopy leaf area index

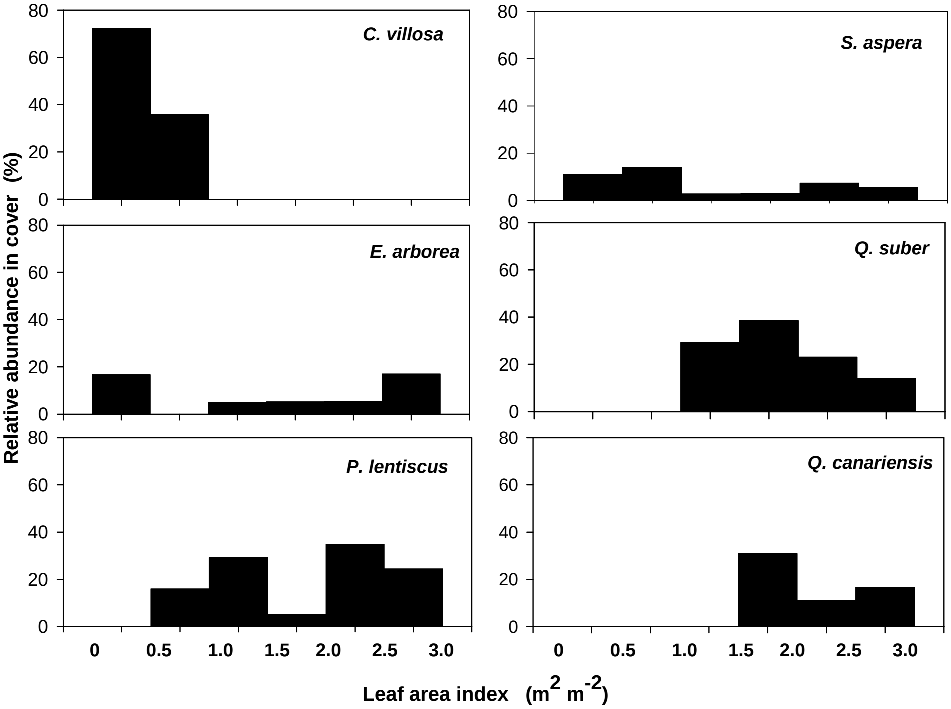

Table S1. Soil properties of the 32 forest plots where leaf samples were collected for leaf trait analysis.

Soil variable	Mean	Range
pH	5.9 ± 0.6	[4.86 - 8.3]
Organic Matter (%)	9.9 ± 2.8	[6.4 - 19.0]
Total N	0.29 ± 0.10	[0.16 - 0.55]
Available nutrients (mg kg^{-1})		
NH_4^+	23.9 ± 14.2	[6.1- 68.5]
P	2.44 ± 1.69	[0.35 - 7.54]
Ca	1781 ± 1214	[396 - 5469]
K	126 ± 72	[46 -297]
Mg	175 ± 79	[53 - 362]
Cu	4.17 ± 3.68	[0.98 - 17.5]
Fe	305 ± 171	[115 - 914]
Mn	402 ± 243	[151 -1149]
S	3.35 ± 0.43	[2.8 - 4.30]
Zn	5.05 ± 4.11	[0.08 -15.86]

Table S2. Abiotic properties of the 60 forest plots where community trait analysis was applied. Data from Pérez-Ramos et al. (2010). GSF, Global Site Factor.

Abiotic Factor	Mean ± SD	Range
Light availability (GSF)	31.7 ± 23.7	[8.0-88.9]
Soil Volumetric Water Content (%)		
Summer	9.3 ± 2.8	[5.0-17.5]
Spring	53.7 ± 22.5	[25.2 - >65]
Soil pH	6.2 ± 0.6	[5.4-8.3]
Soil organic matter (%)	10.5 ± 3.7	[4.8-20.6]
Available nutrients		
NH_4^+ (mg kg^{-1})	30.7 ± 29.1	[6.8-160.9]
P (mg kg^{-1})	1.9 ± 1.8	[0.2-8.9]
Ca (mg kg^{-1})	2903 ± 1749	[477-7925]
K (mg kg^{-1})	191 ± 69	[89-419]
Mg (mg kg^{-1})	261 ± 100	[69-530]

Table S3. Morphological leaf traits of the studied species. Mean ± standard deviation. LMA: Leaf Mass Area; LDMC: Leaf Dry Matter Content.

Species	LMA (g m^{-2})	LDMC (g g^{-1})	Leaf Area (cm^{-2})	Thickness (mm)
C. monogyna	73.2 ± 25.8	0.35 ± 0.1	4.14 ± 1.75	0.23 ± 0.03
C. salvifolius	140 ± 37	0.35 ± 0.05	1.81 ± 0.67	0.47 ± 0.07
C. villosa	72.1 ± 12.0	0.24 ± 0.01	1.12 ± 1.04	*
E. arborea	96.8 ± 22.1	0.40 ± 0.04	0.03 ± 0.01	*
E. scoparia	118 ± 28.3	0.40 ± 0.06	0.03 ± 0.01	*
L. implexa	92.3 ± 22.3	0.31 ± 0.05	9.14 ± 1.59	0.30 ± 0.04
O. alba	115 ± 31	0.30 ± 0.03	0.75 ± 0.32	
P. latifolia	122 ± 29	0.46 ± 0.04	5.55 ± 1.20	0.26 ± 0.04
P. lentiscus	103 ± 21	0.36 ± 0.03	14.2 ± 3.14	0.32 ± 0.07
P. purpurea	125 ± 28	0.38 ± 0.04	14.3 ± 8.9	0.66 ± 0.08
Q. canariensis	116 ± 20	0.46 ± 0.02	20.7 ± 10.5	0.32 ± 0.05
Q. suber	144 ± 24	0.45 ± 0.04	7.62 ± 2.60	0.35 ± 0.04
R. pouzinii	68.1± 5.3	0.39 ± 0.01	23.3 ± 4.2	0.18± 0.01
R. ulmifolius	60.4 ± 5.3	0.40 ± 0.03	43.7 ± 9.20	0.27 ± 0.06
S. aspera	86.2 ± 17.9	0.30 ± 0.02	27.6 ± 5.45	0.30 ± 0.04
T. fruticans	78.7 ± 20.9	0.35 ± 0.05	3.97 ± 1.24	0.33 ± 0.01
T. linifolia	151 ± 44	0.42 ± 0.16	1.20 ± 0.22	*
Community CV (%)	34	21	119	38

*for these species, leaf thickness could not be obtained

Table S4. Macronutrient concentrations in the leaves of the studied species. Mean ± standard deviation. The Coefficient of Variation at the community level (CV) is also indicated .

Sp	C (g kg^{-1})	N_{mass} (g kg^{-1})	N_{area} (g m^{-2})	P_{mass} (g kg^{-1})	P_{area} (g m^{-2})	Ca (g kg^{-1})	K (g kg^{-1})	Mg (g kg^{-1})	S (g kg^{-1})
C. monogyna	485 ± 14	13.5 ± 0.6	0.99 ± 0.35	1.06 ± 0.11	0.078 ± 0.029	18.1 ± 2.8	14,8 ± 1,3	5,08 ± 0,82	1,55 ± 0,14
C. salvifolius	490 ± 7	11.0 ± 1.3	1.52 ± 0.32	0.90 ± 0.12	0.132 ± 0.027	11.7 ± 3.3	7,7 ± 1,5	2,64 ± 0,60	1,18 ± 0,11
C. villosa	481 ± 12	28.9 ± 6.0	2.09 ± 0.58	0.71 ± 0.20	0.051 ± 0.009	18.4 ± 4.4	16,1 ± 5,0	3,98 ± 1,44	1,58 ± 0,40
E. arborea	552 ± 42	12.8 ± 1.4	1.25 ± 0.33	0.59 ± 0.08	0.056 ± 0.014	3.3 ± 0.5	8,3 ± 1,9	2,09 ± 0,37	1,28 ± 0,17
E. scoparia	584 ± 16	13.4 ± 3.1	1.53 ± 0.23	0.67 ± 0.17	0.076 ± 0.009	4.3 ± 0.6	6,6 ± 2,6	2,01 ± 0,42	1,49 ± 0,32
L. implexa	487 ± 11	10.3 ± 2.3	0.94 ± 0.26	0.65 ± 0.15	0.059 ± 0.014	15.8 ± 3.7	23,1 ± 1,9	4,41 ± 0,52	1,51 ± 0,44
O. alba	453 ± 26	18.4 ± 4.8	2.03 ± 0.45	2.73 ± 1.00	0.288 ± 0.086	23.5 ± 5.3	34,5 ± 7,2	4,39 ± 1,35	2,43 ± 0,40
P. latifolia	524 ± 10	14.8 ± 2.1	1.79 ± 0.39	0.95 ± 0.21	0.122 ± 0.044	6.2 ± 1.5	9,5 ± 2,1	1,51 ± 0,45	1,36 ± 0,32
P. lentiscus	515 ± 19	13.9 ± 1.2	1.44 ± 0.30	1.14 ± 0.29	0.136 ± 0.030	6.1 ± 2.0	12,3 ± 2,5	1,45 ± 0,25	1,50 ± 0,56
P. purpurea	484 ± 11	12.6 ± 1.9	1.54 ± 0.29	1.15 ± 0.16	0.155 ± 0.023	9.9 ± 4.2	19,5 ± 4,1	1,45 ± 0,37	1,21 ± 0,21
Q. canariensis	497 ± 15	16.9 ± 2.0	1.98 ± 0.50	0.96 ± 0.20	0.115 ± 0.020	9.0 ± 2.2	9,7 ± 1,8	1,60 ± 0,45	1,36 ± 0,13
Q. suber	516 ± 7	12.1 ± 1.4	1.73 ± 0.22	0.99 ± 0.08	0.150 ± 0.022	2.9 ± 0.4	7,7 ± 0,9	1,31 ± 0,14	0,94 ± 0,05
R. pouzinii	476 ± 15	13.6 ± 2.2	1.12 ± 0.46	0.91 ± 0.12	0.078 ± 0.019	16.8 ± 2.4	11,9 ± 3,1	2,97 ± 0,80	1,34 ± 0,11
R. ulmifolius	487 ± 10	16.8 ± 2.1	1.02 ± 0.46	1.00 ± 0.15	0.060 ± 0.025	11.3 ± 1.6	13,9 ± 1,4	4,91 ± 0,83	1,46 ± 0,26
S. aspera	517 ± 8	11.1 ± 1.4	1.02 ± 0.17	1.11 ± 0.42	0.100 ± 0.035	6.3 ± 2.1	15,5 ± 5,1	1,53 ± 0,66	1,60 ± 0,45
T. fruticans	487 ± 15	18.6 ± 2.5	0.95 ± 0.24	0.93 ± 0.22	0.072 ± 0.007	20.3 ± 8.5	17,4 ± 5,9	1,97 ± 0,44	1,75 ± 0,18
T. linifolia	516 ± 3	25.6 ± 2.1	3.78 ± 1.11	0.79 ± 0.07	0.122 ± 0.045	11.6 ± 2.9	18,8 ± 1,2	1,86 ± 0,24	1,53 ± 0,15
Community CV (%)	6.7	35	44	54	58	60	52	55	27

Table S5. Micronutrient concentrations (mg kg^{-1})in the leaves of the studied species. Mean ± standard deviation. The Coefficient of Variation at the community level (CV) is also indicated.

Sp	B	Ba	Fe	Cd	Co	Cu	Mn	Ni	Pb	Zn
C. monogyna	44.4 ± 13.7	86.9 ± 20.7	131 ± 44	0.144 ± 0.323	0.263 ± 0.254	13.9 ± 13.2	107.7 ± 34.3	1.51 ± 0.31	0.60 ± 0.84	40.0 ± 16.2
C. salvifolius	51.6 ± 8.5	32.9 ± 15.3	171 ± 31	0.229 ± 0.230	1.222 ± 1.287	8.7 ± 7.6	202.2 ± 195.4	3.46 ± 1.57	0.25 ± 0.45	70.8 ± 40.1
C. villosa	42.4 ± 25.2	15.4 ± 12.3	184 ± 50	0.000 ± 0.000	0.157 ± 0.221	55.9 ± 43.3	543.9 ± 160.2	7.17 ± 3.09	1.97 ± 2.30	58.2 ± 36.1
E. arborea	46.8 ± 16.0	17.0 ± 4.5	133 ± 75	0.007 ± 0.012	0.086 ± 0.045	17.7 ± 9.0	105.8 ± 50.4	2.47 ± 0.75	0.99 ± 0.42	21.3 ± 5.4
E. scoparia	54.4 ± 20.3	9.5 ± 1.7	74 ± 28	0.117 ± 0.262	0.032 ± 0.041	13.4 ± 12.9	23.3 ± 9.8	1.28 ± 0.55	0.55 ± 0.68	21.1 ± 9.8
L. implexa	38.0 ± 12.1	34.0 ± 7.3	97 ± 28	0.054 ± 0.029	0.059 ± 0.006	46.1 ± 36.3	71.3 ± 26.3	1.87 ± 1.25	2.38 ± 1.86	51.5 ± 35.3
O. alba	37.4 ± 22.2	40.1 ± 25.3	58 ± 13	0.081 ± 0.017	0.344 ± 0.140	23.8 ± 7.5	927.1 ± 319.0	8.20 ± 2.81	0.46 ± 0.42	37.4 ± 16.8
P. latifolia	14.6 ± 4.5	11.8 ± 3.9	59 ± 22	< 0.001	0.065 ± 0.132	22.6 ± 15.5	29.8 ± 5.3	3.33 ± 0.80	0.83 ± 0.86	44.1 ± 15.2
P. lentiscus	23.2 ± 8.9	19.1 ± 11.3	54 ± 26	< 0.001	< 0.001	36.3 ± 21.1	52.5 ± 25.8	1.04 ± 0.49	1.68 ± 1.24	32.4 ± 14.7
P. purpurea	55.6 ± 22.4	12.3 ± 4.4	193 ± 44	< 0.001	0.016 ± 0.021	11.9 ± 5.8	63.9 ± 20.3	1.67 ± 1.00	0.26 ± 0.34	21.8 ± 5.3
Q. canariensis	47.0 ± 9.9	43.0 ± 15.9	114 ± 11	< 0.001	0.046 ± 0.052	17.2 ± 6.7	643.7 ± 203.9	3.43 ± 0.83	0.69 ± 0.41	25.5 ± 7.1
Q. suber	25.1 ± 2.5	7.0 ± 2.2	77 ± 19	< 0.001	0.111 ± 0.119	19.2 ± 9.7	363.0 ± 69.2	3.42 ± 0.94	0.73 ± 0.61	23.5 ± 4.7
R. serpervirens	61.6 ± 14.6	23.3 ± 12.8	100 ± 23	0.008 ± 0.008	0.066 ± 0.010	13.1 ± 5.1	121.9 ± 50.7	1.85 ± 0.35	0.67 ± 0.30	19.9 ± 3.8
R. ulmifolius	43.8 ± 13.4	26.6 ± 6.9	171 ± 40	0.007 ± 0.002	0.173 ± 0.069	15.7 ± 10.7	245.2 ± 85.5	2.88 ± 0.70	0.79 ± 0.58	31.1 ± 9.9
S. aspera	22.9 ± 11.1	18.8 ± 24.2	66 ± 26	< 0.001	0.000 ± 0.000	33.8 ± 32.7	86.1 ± 85.8	1.27 ± 0.87	1.59 ± 1.85	35.7 ± 20.8
T. fruticans	29.5 ± 4.0	33.2 ± 10.6	135 ± 17	0.070 ± 0.052	0.162 ± 0.088	56.7 ± 32.0	193.5 ± 104.1	5.13 ± 0.75	2.73 ± 1.74	62.5 ± 30.7
T. linifolia	28.6 ± 3.5	21.4 ± 6.5	165 ± 23	0.004 ± 0.007	0.115 ± 0.092	6.0 ± 4.3	313.8 ± 57.1	2.70 ± 0.75	0.33 ± 0.28	36.6 ± 1.7
Community CV (%)	47	83	48	291	235	98	111	74	119	63

Table S6. Abundance of ^{15}N and ^{13}C in the leaf of the studied species

	δ^{15}N	δ^{13}C
C. monogyna	-3.23 ± 0.74	-30.7 ± 0.9
C. salvifolius	-2.52 ± 0.92	-29.2 ± 0.9
C. villosa	0.44 ± 0.72	-30.4 ± 0.8
E. arborea	-0.97 ± 1.41	-28.6 ± 1.1
E. scoparia	-0.91 ± 1.54	-30.4 ± 1.6
L. implexa	-3.66 ± 0.53	-29.5 ± 1.6
O. alba	0.38 ± 0.94	-29.0 ± 0.2
P. latifolia	-4.15 ± 0.42	-29.1 ± 1.7
P. lentiscus	-2.88 ± 0.97	-29.6 ± 1.2
P. purpurea	-2.70 ± 0.77	-28.5 ± 1.1
Q. canariensis	-0.49 ± 0.42	-28.3 ± 0.8
Q. suber	-0.83 ± 0.13	-28.5 ± 0.5
R. serpervirens	-2.59 ± 0.98	-29.2 ± 0.5
R. ulmifolius	-3.14 ± 1.14	-30.5 ± 0.5
S. aspera	-0.72 ± 0.66	-27.3 ± 1.2
T. fruticans	-2.05 ± 1.95	-29.5 ± 1.5
T. linifolia	0.08 ± 0.31	-29.5 ± 1.1
Community range	[-4.14, 0.43]	[-30.7, -27.3]

Table S7. Result of the Principal Component Analysis (factor-variable correlations) applied to the morphological and the chemical variables

	Factor 1	Factor 2
LMA	0.58	-0.40
LDMC	0.57	-0.28
C	0.54	-0.39
N	-0.74	-0.55
C:N	0.75	0.36
B	-0.21	0.16
Ca	-0.65	0.41
Co	-0.24	0.22
Cu	-0.41	0.11
Fe	-0.50	-0.10
K	-0.43	0.32
Mg	-0.61	0.42
Mn	-0.45	-0.53
N:P	-0.54	-0.69
Ni	-0.64	-0.43
P	0.03	0.44
S	-0.52	0.17
Zn	-0.49	0.26
δ^{13}C	0.50	-0.17
δ^{15}N	-0.12	-0.69

Table S8. Summary of the SMA correlation results between the Leaf Mass per Area and the rest of studied leaf traits. For those significant relationships (r^2 in bold) slope p-values are also indicated. Those slope values with a p-value < 0.05 were significantly different than 1.

Variable	r^2	*P* -value	Slope				Intercept
			Value	Lower CI	Upper CI	p-value	
N_{mass}	0.01	0.65	-1.01	-1.70	-0.60		
N_{area}	**0.39**	0.01	1.27	0.84	1.93	0.25	-2.37
N_{mass}*	0.06	0.378	-0.68	-1.191	-0.39		
N_{area}*	**0.54**	0.002	0.98	0.66	1.47	0.95	-1.84
C:N	0.03	0.49	1.07	0.64	1.80		
$\delta^{15}N$	0.00	0.84	3.86	2.28	6.52		
$\delta^{13}C$	**0.25**	0.04	**4.21**	2.66	6.67	< 0.001	-8.45
P_{mass}**	0.00	0.95	-0.72	-1.25	-0.42		
P_{area}**	**0.56**	0.00	1.31	0.90	1.89	0.15	-3.65
Ca_{mass}	0.17	0.10	-2.24	-3.63	-1.38		
Ca_{area}	0.00	0.99	2.01	1.19	3.41		
Fe_{mass}	0.03	0.51	-1.55	-2.61	-0.92		
K_{mass}	0.05	0.39	-1.56	-2.61	-0.93		
K_{area}	0.13	0.15	1.61	0.99	2.64		
Mg_{mass}	**0.36**	0.01	**-1.70**	-2.61	-1.11	0.02	3.77
Mg_{area}	0.00	0.93	-1.35	-2.28	-0.80		
S_{mass}	0.11	0.19	-0.69	-1.14	-0.42		
S_{area}	**0.56**	0.00	0.95	0.66	1.35	0.76	-2.74
Ba_{mass}	0.14	0.14	-2.20	-3.60	-1.35		
Co_{mass}	0.05	0.39	2.31	1.38	3.86		
Cu_{mass}	0.19	0.09	-2.24	-3.62	-1.39		
Mn_{mass}	0.00	0.98	-3.73	-6.32	-2.21		
Ni_{mass}	0.00	0.87	2.10	1.24	3.55		
Pb_{mass}	**0.28**	0.03	**-2.59**	-4.06	-1.65	< 0.001	5.08
Zn_{mass}	0.01	0.76	-1.43	-2.41	-0.85		

* excluding N-fixing species from the data set

** excluding hemiparasitic species (*Osyris alba*) from the data set

Table S9. Transfer quotients (leaf : soil concentration ratios) of macro and micronutrients in the studied species (mean ± standard deviation)

Sp	Ca	K	N	Mg	P	S	Cu	Fe	Mn	Zn
C. monogyna	19.3 ± 4.8	167 ± 100	648 ± 208	34.9 ± 11.6	324 ± 113	511.4 ± 14.4	8.2 ± 11.4	0.45 ± 0.10	0.37 ± 0.28	6.3 ± 3.3
C. salvifolius	13.4 ± 8.8	97 ± 64	485 ± 99	21.9 ± 9.1	427 ± 268	386.8 ± 34.2	3.5 ± 4.5	0.71 ± 0.43	0.52 ± 0.44	18.5 ± 9.8
C. villosa	12.3 ± 6.8	193 ± 105	2821 ± 2735	27.3 ± 19.8	364 ± 318	479.3 ± 137.1	15.7 ± 11.3	0.72 ± 0.21	1.93 ± 1.25	17.7 ± 15.5
E. arborea	3.0 ± 1.8	85 ± 62	676 ± 431	15.6 ± 8.5	474 ± 434	388.5 ± 90.8	7.9 ± 2.7	0.83 ± 0.72	0.41 ± 0.47	10.9 ± 13.7
E. scoparia	5.0 ± 3.7	110 ± 80	936 ± 473	20.4 ± 12.6	354 ± 208	449.7 ± 50.2	3.0 ± 1.3	0.26 ± 0.11	0.08 ± 0.03	6.3 ± 3.7
L. implexa	10.1 ± 4.1	255 ± 150	890 ± 503	31.1 ± 19.1	544 ± 303	390.0 ± 47.1	9.0 ± 7.0	0.40 ± 0.27	0.14 ± 0.09	17.8 ± 17.4
P. latifolia	4.5 ± 2.1	114 ± 54	756 ± 304	11.6 ± 5.9	435 ± 225	361.0 ± 87.3	6.3 ± 4.4	0.21 ± 0.13	0.10 ± 0.05	9.9 ± 9.7
P. lentiscus	7.0 ± 2.1	183 ± 59	807 ± 322	13.0 ± 3.7	481 ± 307	473.5 ± 189.2	12.8 ± 10.0	0.26 ± 0.11	0.16 ± 0.12	9.6 ± 6.3
P. purpurea	9.8 ± 4.1	264 ± 172	605 ± 232	13.7 ± 6.5	834 ± 775	371.8 ± 88.5	2.9 ± 2.5	0.89 ± 0.59	0.15 ± 0.12	5.8 ± 4.8
Q. canariensis	11.2 ± 5.1	157 ± 43	993 ± 333	14.2 ± 5.2	276 ± 163	404.9 ± 55.1	9.1 ± 5.3	0.55 ± 0.22	2.63 ± 0.90	5.8 ± 2.9
Q. suber	4.1 ± 1.2	143 ± 25	638 ± 247	13.7 ± 2.1	357 ± 176	295.4 ± 27.7	9.2 ± 4.5	0.40 ± 0.10	1.53 ± 0.62	8.5 ± 2.5
R. serpervirens	13.2 ± 5.9	160 ± 101	1122 ± 776	24.3 ± 11.9	809 ± 824	379.5 ± 20.2	3.3 ± 1.6	0.45 ± 0.15	0.42 ± 0.20	57.1 ± 114.8
R. ulmifolius	11.2 ± 4.4	210 ± 93	1157 ± 910	43.6 ± 19.0	372 ± 194	478.4 ± 44.7	7.6 ± 6.8	0.98 ± 0.37	0.86 ± 0.33	10.8 ± 4.8
S. aspera	5.8 ± 5.3	180 ± 94	701 ± 384	12.1 ± 6.1	540 ± 138	379.6 ± 50.5	12.9 ± 14.9	0.24 ± 0.13	0.30 ± 0.33	8.9 ± 7.5
T. fruticans	15.6 ± 5.8	242 ± 142	1176 ± 118	13.6 ± 2.5	326 ± 103	516.3 ± 18.8	17.9 ± 19.3	0.66 ± 0.42	0.65 ± 0.60	11.5 ± 10.9
T. linifolia	9.8 ± 3.2	241 ± 106	2560 ± 2056	12.7 ± 4.4	589 ± 525	462.6 ± 50.1	1.6 ± 1.9	0.59 ± 0.18	0.96 ± 0.66	9.7 ± 9.2
Community CV	47	49	45	54	118	25	47	49	45	54

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Table S10. Summary of the SMA correlation results between the Leaf Mass per Area and the nutrient transfer quotients. For those significant relationships, slope p-values are also indicated. Those slope values with a p-value < 0.05 were significantly different than -1.

Element	r^2	p -value	Slope				Intercept
			Value	Lower CI	Upper CI	p-value	
Ca	0.24	0.066	-1.78	-2.88	-1.10		
Cu	**0.39**	0.030	**-2.08**	-3.21	-1.34	< 0.001	4.98
K	0.15	0.281	-0.90	-1.50	-0.54		
Fe	0.05	0.445	-1.61	-2.74	-0.94		
N	0.09	0.319	-1.51	-2.55	-0.90		
N*	**0.37**	0.022	-0.97	-1.57	-0.60	0.90	4.84
Mg	**0.61**	0.002	-1.36	-1.93	-0.95	0.09	3.98
Mn	0.01	0.706	-3.71	-6.39	-2.16		
P	0.09	0.761	0.31	0.18	0.52		
S	**0.38**	0.026	**-0.47**	-0.73	-0.30	< 0.001	3.56
Zn	0.35	0.100	-1.43	-2.24	-0.92		

* excluding N-fixing species from the data set

Table S11. Results of the bivariate correlations between aggregated community traits. Bold characters indicate significant values. LMA$_{CWM}$, Leaf Mass Area; LDMC$_{CWM}$, Leaf Dry Matter Content. * p *<0.05*; ** p <0.01; *** p<0.001.

	LMA$_{CWM}$	LDMC$_{CWM}$	C$_{CWM}$	N$_{CWM}$	C:N$_{CWM}$	^{13}C$_{CWM}$
LDMC$_{CWM}$	**0.83 *****					
C$_{CWM}$	**0.32 ***	**0.35 ****				
N$_{CWM}$	**-0.75 *****	**-0.71 *****	**-0.43 *****			
C:N$_{CWM}$	**0.70 *****	**0.58 *****	**0.61 *****	**-0.95 *****		
δ^{13}C$_{CWM}$	**0.56 *****	**0.59 *****	0.01	**-0.79 *****		
δ^{15}N$_{CWM}$	**-0.32 ***	**-0.41 ****	0.04	**0.60 *****	**-0.48 *****	**-0.39 ****

Table S12. Results of the bivariate correlations between aggregated community traits and environmental factors. Values significant on a per-test basis (p< 0.05) are in italic. Bold characters indicate significant values after controlling for the false discovery rate. LMA, Leaf Mass Area; LDMC, Leaf Dry Matter Content; GSF. Global Site factor; LAI, Leaf Area Index. * p *<0.05*; ** p <0.01; *** p<0.001.

	LMA_{CWM}	$LDMC_{CWM}$	C_{CWM}	N_{CWM}	$C:N_{CWM}$	$\delta^{13}C_{CWM}$	$\delta^{15}N_{CWM}$
LAI	***0.49****	***0.66****	*0.31*	***-0.55****	***0.47****	***0.45****	***-0.36***
GSF	***-0.63****	***-0.78****	*-0.32*	***0.66****	***-0.56****	***-0.53****	***0.37***
Soil pH	-0.08	-0.08	***-0.39***	-0.03	-0.05	0.24	-0.22
Soil OM	0.01	0.03	*-0.27*	-0.16	0.09	0.23	***-0.39***
Total N	-0.01	0.03	-0.20	-0.16	0.09	0.21	***-0.34***
Soil NH_4^+	0.18	0.16	0.08	-0.20	0.19	0.14	-0.09
Soil P	*0.32*	*0.28*	0.23	-0.23	0.25	0.09	0.02
Soil Ca	0.02	-0.05	***-0.39***	-0.13	0.05	*0.32*	*-0.33*
Soil K	0.08	-0.05	-0.17	-0.16	0.15	0.19	-0.15
Soil Mg	0.23	*0.26*	-0.18	-0.21	0.11	*0.29*	-0.14
Spring soil moisture	-0.16	-0.20	***-0.37***	0.01	-0.06	0.25	-0.12
Summer soil moisture	-0.04	-0.02	***-0.37***	-0.12	0.02	***0.35***	-0.22

Capítulo 4.

El retorno de nutrientes a través de la hojarasca y sus efectos en las condiciones del suelo

Este capítulo reproduce el siguiente manuscrito:

Aponte, C., García, L.V., Pérez-Ramos, I.M., Gutiérrez, E., Marañón, T. *Oak trees and soil interactions: a positive feedback model*. Enviado a Journal of Vegetation Science (Octubre 2010)

Capítulo 4. **El retorno de nutrientes a través de la hojarasca y sus efectos en las condiciones del suelo**

RESUMEN

Las especies arbóreas pueden modificar distintamente las condiciones del suelo y estos cambios pueden a su vez favorecer el reclutamiento de plántulas de las propias especies dando lugar a un efecto de retroalimentación positiva. En este trabajo hemos estudiado las interacciones planta-suelo establecidas por dos quercíneas de hábito foliar contrastado, el alcornoque (*Quercus suber*) de hoja perenne y esclerófila, y el quejigo moruno (*Q. canariensis*) de hoja caduca y marcescente, en los bosques del sur de España. Para ello examinamos el contenido en macro y micro nutrientes en cinco componentes del ecosistema: la hoja viva, la hoja de desfronde, la hojarasca del suelo y el suelo superficial (0-25 cm) y sub-superficial (25-50 cm) bajo la copa de 50 individuos.

La variabilidad en la concentración de elementos químicos estuvo relacionada con la naturaleza de los ciclos biogeoquímicos y del tipo de enlace establecido con compuestos orgánicos. La concentración de estos elementos químicos fue consistente en todos los componentes del ecosistema analizados. Se utilizó un análisis de co-inercia para investigar los patrones multivariantes de covariación de los elementos químicos en el ecosistema. Los resultados mostraron la existencia de un gradiente principal de enriquecimiento de nutrientes común a todos los componentes. Ambas especies de quercíneas ocuparon extremos opuestos del gradiente de fertilidad, siendo los tejidos y el suelo bajo *Q. canariensis* más ricos en nutrientes que los de *Q. suber*. Empleando un análisis de modelos causales se validó un modelo teórico de retroalimentación positiva según el cual ambas especies modifican distintamente las condiciones de fertilidad del suelo mediante un retorno diferente de nutrientes. Estos cambios generados en el suelo podrían, a su vez, afectar al establecimiento de plántulas y, finalmente, influir en la distribución de las propias especies.

Oak trees and soil interactions: a positive feedback model

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Abstract

Coexisting tree species with different leaf chemical compositions and varying nutrient return to the soil may distinctly modify soil conditions and create a mosaic of fertility that reflects canopy composition. Soil conditions may affect seedling recruitment and the long-term species distribution, resulting in a positive feedback effect. We investigated the tree-soil interactions of two contrasting oak species in the forests of southern Spain. We examined five different ecosystem components of the evergreen Quercus suber and the deciduous Q. canariensis: leaves, leaf fall, litter, superficial soil (0-25 cm) and sub-superficial soil (25-50 cm). We sampled and determined the content of macronutrients and micronutrients of the leaves, leaf fall, and litter of 50 selected trees. We also studied chemical properties (nutrient content and availability) of the superficial soil and sub-superficial soil beneath the canopy of each tree.

Variability in the concentration of chemical elements was related to the nature of their biogeochemical cycles and whether they were bound to organic compounds. However, the concentration of chemical elements was consistent across ecosystem components. We used a multiple analysis of co-inertia to investigate the multivariate patterns of covariation in element concentration throughout the forest ecosystem. The results revealed the existence of a main gradient of nutrient enrichment common to all ecosystem components. Both oak species were statistically separated along the nutrient fertility gradient, with Q. canariensis having richer plant tissues and more fertile soil directly under each tree than Q. suber.

A theoretical tree-soil positive feedback model was validated using a path analysis. In this model, oak species distinctly modify soil fertility conditions through different nutrient return pathways. These tree-generated soil changes could, in turn, affect seedling establishment and might ultimately affect species distribution.

Keywords*: Quercus suber, Mediterranean forest, nutrient cycling, ecosystem analysis, foliar analysis, ecological stoichiometry, soil fertility*

INTRODUCTION

Ecosystem function and stability relies on ecosystem interactions. Understanding the complexity and the underlying mechanisms of plant-soil feedback effects is crucial to understanding ecosystems and their responses to changing environmental conditions due to global climate change (Ehrenfeld et al., 2005). In forests ecosystems, trees play a key role in these feedback effects because of their capacity to modify soil properties and nutrient cycling (Finzi et al., 1998; Lovett et al., 2004). In turn, soil characteristics influence tree seedling establishment, survivorship and tree species distribution (Goldberg, 1982; Kobe et al., 1995). Potential positive feedback effects occur when soil property changes that are generated by a tree species promote recruitment and increase the fitness of seedlings of the same species (Ehrenfeld et al., 2005). These feedback effects have ecological and evolutionary consequences for tree populations (Binkley and Giardina, 1998; van Breemen and Finzi, 1998) and are key to understanding and predicting forest dynamics (Weand et al., 2010).

Tree species have a characteristic mineral element composition in leaves, stems and roots. This is a multivariate phenotypic trait that reflects the genotypic and environmental interactions influencing the chemical content of plants (Garten, 1978). The species position in the multivariate space of elemental concentration has been defined as a species’ "biogeochemical niche" and might reflect differences in the use of nutrients (Peñuelas et al., 2008). Most studies on chemical element variability and species’ biochemical niches have been restrained to foliar tissues (Alonso and Herrera, 2001, 2003; Watanabe et al., 2007), whereas only few studies have analyzed two distinct ecosystem components (i.e. leaves and soil) (Merila and Derome, 2008). The biogeochemistry of chemical elements depends on their biological control, chemical bonding properties, and the source of elements (Ladanai et al., 2010). The biochemical traits (i.e., mineral content and stoichiometry) of leaves determine the physical and chemical characteristics of leaf fall. Furthermore, they influence leaf decomposition and mineralization rates and the incorporation of organic matter and nutrients into the soil. Through distinct nutrient return and the associated changes in soil acidity and nutrient availability, different tree species can modify soil fertility and nutrient cycling and potentially increase the fitness of individuals of the same species (Hobbie, 1992; Finzi et al., 1998; Gómez-Aparicio and Canham, 2008). In addition, these changes in soil fertility and acidity can consequently alter the habitat and resources of soil organisms. Soil communities have a strong influence on the physical properties (i.e., aireation) and chemical properties of soil because they control decomposition and mineralization processes (Wardle et al., 2004). Thus, changes in soil properties generated by trees could shift the abundance and composition of soil communities (Aponte et al., 2010) and result in changes in the plant community (Mitchell et al., 2007).

Most studies of tree-soil interactions have been conducted in temperate and boreal forests (Knops et al., 2002; Mitchell et al., 2007; Talbot and Finzi, 2008), whereas equivalent studies in Mediterranean forests are less frequent (Gallardo and Merino, 1993). Mediterranean forests are subjected to marked climate seasonality, with warm, dry summers that drive ecosystem dynamics. Changes in climate conditions will affect the ecosystem processes, the availability of resources and species' performance (Bates et al., 2008; van Meeteren et al., 2008). In general, forests in the Mediterranean region are particularly vulnerable to global change (Schröter et al., 2005). Unveiling the mechanism of tree-soil interactions will contribute to reducing the uncertainties of the influence of future climate change on Mediterranean-type ecosystems.

We aimed to investigate the tree-soil feedback effect in Mediterranean forests by comparing two distinct but coexisting oak species, the evergreen cork oak (*Quercus suber*) and the winter deciduous *Q. canariensis*. Trees with long-lived leaves (i.e., evergreens) tend to occupy nutrient-poor environments and are characterized by their conservative use of nutrients compared to deciduous trees with short-lived leaves (Aerts, 1995). Therefore, it is expected that biogeochemical interactions of these trees with soil should be different.

We studied five different components of the ecosystem: living leaves, leaf fall, litter, superficial soil and sub-superficial soil. These five components were studied at three different forest sites. Specifically, we pursued four main objectives. First, we aimed to analyze the relationships among nutrients within each compartment of the ecosystem to understand the variability and correlational patterns of chemical elements in the ecosystem. We expected to find less variability for elements that are organically bound and subjected to tighter biological control and more variable stoichiometry for non-limiting mineral elements. Second, we extracted the main multivariate patterns of nutrient covariation within and between the ecosystem compartments. We hypothesized that the transfer of nutrients across leaves, leaf fall, litter and soil would be reflected in a common structure of nutrient covariation in the ecosystem. Third, we aimed to ascertain whether the two co-occurring oak species, *Quercus canariensis* and *Q. suber,* occupy different biogeochemical niches within the ecosystem. We expected differences in their position in the multivariate space defined by their tissue and soil nutrient composition given the differences in their leaf habits. Last, we evaluated whether there is a causal chain that links the ecosystem compartments in a tree-soil feedback cycle with a d-sep method. We hypothesized that both oak species distinctly modify soil properties through their contrasted chemical traits and that these changes might affect seedling establishment and ultimately affect species distribution.

METHODS

Study area

The study area was located in the Aljibe Mountains in southern Spain. Acidic and nutrient-poor soils (Palexeralfs) exist on an Oligo-Miocene sandstone bedrock that is interspersed with layers of marl sediments, yielding soils richer in clay (Haploxererts; nomenclature according to Soil Survey Staff, 2010). The area has a sub-humid Mediterranean climate, with a dry and warm summer period of 3-4 months and most rainfall (95%) occurring from October to May. The annual rainfall varies from 701 mm to 1331 mm, and annual mean temperatures range from 14.6 ºC in the most elevated areas to 18.4 ºC in the coastal zones (Anonymous, 2005). The dominant vegetation is a mixed forest of evergreen cork oak (*Quercus suber* L.) and winter-deciduous Algerian oak (*Q. canariensis* Willd). The arborescent shrubs *Phillyrea latifolia* L. and *Pistacia lentiscus* L. dominate the understory (Ojeda et al., 2000). The area has been protected since 1989 as Los Alcornocales ("the cork oak forests") Natural Park. Three structurally different forest sites (30 km apart) were selected within the study area. These sites were San Carlos del Tiradero, Buenas Noches and La Sauceda. Table 1 presents the characteristics of the climate, soil and vegetation of these study sites (see details in Quilchano et al. 2008, and Pérez-Ramos et al. 2008).

Sampling design

At each site, ten individuals of *Q. suber* and ten individuals of *Q. canariensis* were selected within a 1-ha mixed forest stand, with the exception of the Buenas Noches site, where the forest stand was monospecific, and thereby only ten individuals of *Q. suber* could be selected. All trees (a total of 30 *Q. suber* and 20 *Q. canariensis*) were estimated to be more than 50 years old.

The five ecosystem components, leaves, leaf fall, litter, superficial soil (0-25 cm depth) and sub-superficial soil (25-50 cm depth), were sampled in November 2006. Fully expanded leaves were sampled at each of the four cardinal points for each tree. Leaf fall, litter and soil were sampled beneath the canopy of each selected oak at approximately 2 m from the trunk. Annual leaf fall was collected by four traps (50 cm diameter) located under each tree. The contents were removed and the leaf fraction was separated and dried. Litter was harvested using two 30 x 30-cm quadrats. Soil cores were extracted with a cylindrical auger. Four samples of superficial soil (0-25 cm) and four samples of sub-superficial soil (25-50 cm) were taken under each oak tree and pooled into single representative samples.

Tabla 1. Characteristics of the three studied forest sites. Sources are Pérez-Ramos et al. (2008) and Quilchano et al. (2008).

	San Carlos de Tiradero	Buenas Noches	La Sauceda
Coordinates	36° 9' 46" 5° 35' 39"	36° 22' 56" 5° 34' 57"	36° 31' 54" 5° 34' 29"
Altitude range (m a.s.l)	335-360	410-450	530-560
Soil texture	loam-sand-clay	loam-sand	loam-clay
Gravel (%)	19.7	24.2	16.1
Sand (%)	55.2	46.3	65.2
Silt (%)	24.5	26.7	20.8
Clay (%)	20.1	26.6	14.0
Soil chemistry			
pH	5.6	6.2	5.2
Organic matter	5.9	7.4	9.3
C/N	9.3	11.8	14.3
Mean rainfall (mm)			
Spring	216.8	227.4	258.9
Summer	21.1	24.2	28.0
Autum	262.3	299.2	319.2
Winter	472.2	485.6	526.4
Total	964.5	1027.5	1117.6
Mean temperature (ºC)			
Annual	16.6	16.5	15.5
Minimum	10.9	10.5	9.1
Maximum	23.4	24.0	23.6
Vegetation structure			
Density of trees (stems.ha^{-1})	768.8	400.0	218.8
Density of arborescent shrubs (stems.ha^{-1})	256.3	1156.3	450.0
Basal area(m^2 ha^{-1})	48.3	27.6	24.0
Leaf area index (m^2 m^{-2})	2.26	1.64	1.84

Laboratory analyses

All samples of plant material were dried at 70ºC for 48 h and were then ground for chemical analysis. Soil samples were air dried at 30ºC in a forced air oven and sieved (mesh width: 2 mm). Soil acidity (pH) was determined potentiometrically in a 1:2.5 soil:CaCl solution. The percentage of soil carbon was estimated using a total organic carbon analyzer (TOC-Vesh). The available soil P was estimated using the Bray-Kurtz method. Soil NH_4^+ was extracted with KCl (2 M) and determined by steam-distillation. Available soil Ca, Mg and K were extracted using neutral 1 M ammonium acetate and were then assessed using atomic absorption spectroscopy. Available micronutrients (Mn, Cu and Zn) were extracted using a neutral 0.05 M EDTA solution and were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES). The total concentration of several nutrients (Ca, K, Mg, P, S, Mn, Cu and Zn) in plant tissues and soils was determined by acid digestion followed by ICP-OES analysis. Plant and soil N was determined by Kjeldahl digestion and subsequent distillation–titration in a Bran-Luebbe autoanalyzer. Concentrations of the elements are provided on a dry weight basis. See detailed methods in Jones and Case (1990) and Sparks (1996).

Statistical analysis

The coefficient of variation (CV = standard deviation/mean, expressed as %) was used to examine the variation of chemical elements in the five studied components: leaves, leaf fall, litter, superficial soil and sub-superficial soil. Spearman's correlation coefficients were calculated to investigate relationships among variables within and between subsystems. Differences in the chemical elements between oak species were tested using ANOVA. To control for the type I error inflation derived from the large number of correlations calculated, we used the false discovery rate (FDR) procedure recommended by García (2003).

The covariation of chemical elements in leaves, leaf fall, litter and soil was investigated using a multiple co-inertia analysis (MCoA). This is a multi-table technique that enables the simultaneous ordination of various data sets. MCoA maximizes the variance within each individual table and the correlation between the scores of each individual table (individual ordination) and synthetic scores (ordination of the juxtaposed tables) to provide a reference structure. MCoA thus, shows the covariance between several individual ordinations and a reference ordination that can be viewed as the common structure shared by the individual ordinations. Mathematical details are provided by Chessel and Hanafi (1996).

MCoA was performed on the five datasets containing the chemical element concentrations of leaves, leaf fall, litter, superficial, and sub-superficial soil. Preliminary analysis of the datasets showed that the forest site had a significant effect on most variables and accounted for large variability of the data that distorted the relationships among ecosystem components. To remove the effect of this covariable and obtain comparable values of nutrient concentrations in plant and soil material from the three forest sites, we performed a simple linear regression of each variable. This linear regression used site as a unique predictor and allowed us to subsequently work with the regression residuals. MCoA statistics were computed with the ade4 library implemented from the R freeware (http://www.r-project.org/). The significance of the extracted axes was evaluated using the Broken-stick method (King and Jackson, 1999). Differences between species on the extracted ordination axes were tested using analysis of variance (one-way ANOVA).

We used a path analysis to investigate the causal relationships among the chemical properties of the ecosystem components (Shipley, 2000). We hypothesized a tree-soil feedback model based on previous studies and on the empirical patterns derived from the multiple co-inertia analysis. We also tested several alternative models. These models imply a series of causal assumptions that condition the structure of the variances and covariances of the variables and can be tested against the observed data. A model fits the data significantly when these assumptions cannot be falsified ($p > 0.05$). Otherwise, the model is rejected (when $p < 0.05$) as a feasible explanation of the overall observed relationships between the variables.

RESULTS

Variability of chemical elements in leaves, leaf fall and litter

The average nutrient content of the leaves, leaf fall and litter of the two studied species is presented in Appendix 1. A similar ranking of element concentrations was found for all plant materials, independent of the site and the oak species. Elements were ranked as: N > K > Ca > Mg > S > P > Mn > Zn > Cu. The main change was observed for K, since its relative abundance decreased from leaves to leaf fall and litter. In general, nutrient concentration in leaves, leaf fall and litter tended to be higher for *Q. canariensis* than for *Q. suber*.

Fig. 1 shows the coefficient of variation of the studied elements for the three components of leaves, leaf fall and litter. In leaves, S, P and N were the least variable elements. The variability of S, P and N in leaves and litter was similar for the two oak species, while *Q. canariensis* tended to have higher S and N variability in leaf fall. Other elements (such as Ca, Mn and K) showed higher variability (30-40%) and larger differences between the two oak species (Appendix 1).

Most elements were significantly positively related within each of the three components: 22, 26 and 22 of the 36 possible correlations (≈ 65%) were significant in leaves, leaf fall and litter, respectively (Appendix 3). Organically bound elements (N, P and S) and macronutrients, such as Ca and Mg, had positive correlations in all of the ecosystem components.

Variability of soil chemical properties

Soil acidity, total nutrient content and available nutrient content in the soil sampled beneath each tree canopy are shown in Appendix 2. Soil beneath *Q. canariensis* tended to be less acidic than the soil beneath *Q. suber* (pH 4.5 vs. 4.1) and tended to have a higher content of N (0.20% vs. 0.16%). Cation exchange capacity (CEC), nutrient availability and total element content were generally higher in the soils under the deciduous *Q. canariensis*. The main exception was found for the available and total K in soil, since values were higher beneath *Q. suber*. Among the soil variables, pH had the lowest CV for

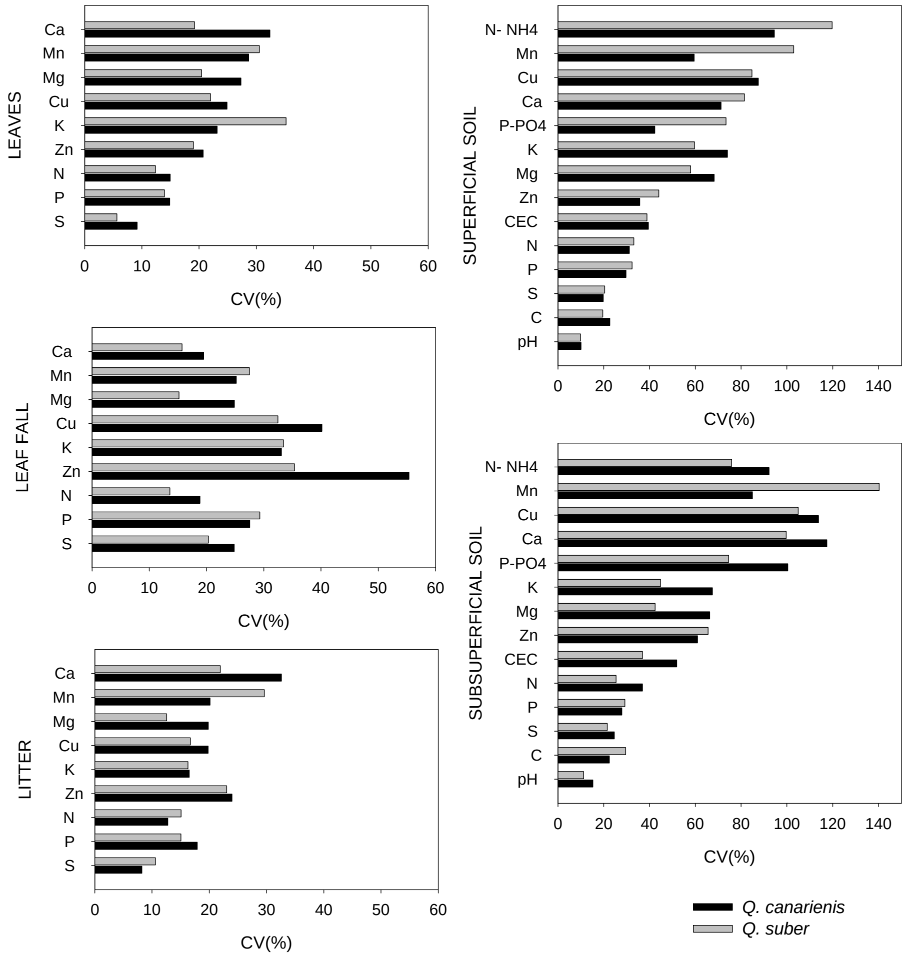

Figure 1. Coefficient of variation (CV) of the chemical elements in the leaves, leaf fall, litter, superficial soil (0-25 cm) and sub-superficial soil (25-50 cm) of the two studied oak species.

both superficial (≈ 10%) and sub-superficial soil (≈ 14%), followed by the organically bound elements C, S, P and N (< 35%) (Fig. 1). Higher variability was recorded for the available and extractable N, P and S than for the total element concentration. Some elements were more variable in the sub-superficial soil (Zn, Ca, Cu and Mn), while others had similar CV in the superficial and sub-superficial soil (Mg and K). Correlations among chemical elements in the soil were higher than in plant tissues: 100% of all possible correlations among total nutrient contents in superficial soil and 86% in the sub-superficial soil were positive and significant (Appendix 3). These significant correlations for the chemical elements in plants and soil material revealed the existence of nutrient enrichment patterns in the ecosystem components.

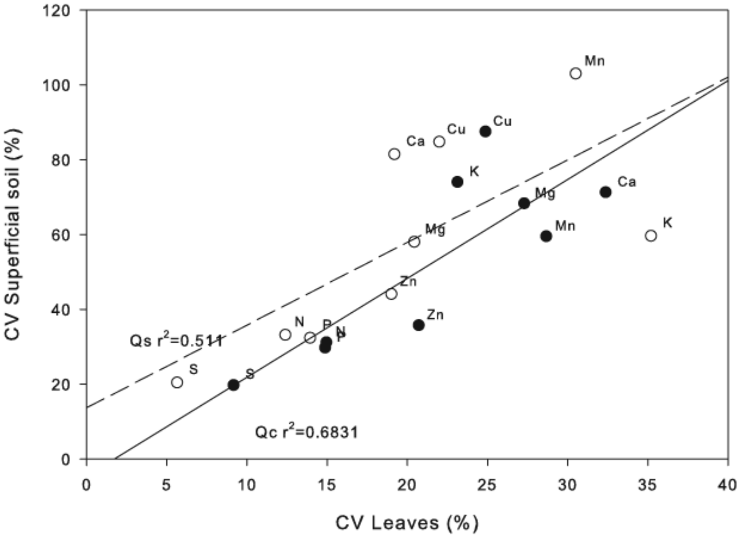

Figure 2. Correlation between the CV values of the nutrient concentrations in leaves and superficial soil for *Q. canariensis* (filled dots, solid regression line) and *Q. suber* (hollow dots, dashed regression line).

Links among ecosystem components

On average, variability in the superficial soil (CV of 55%) and the sub-superficial soil (75%) was higher than in the plant material (20%). When we compared the variability of elements across components, we observed that they were highly related ($r_s > 0.6$); thus, the pattern of nutrient variability was consistent between ecosystem components (Fig. 2). The organically bound nutrients (N, P, S) had the lowest CVs, whereas elements such as Ca and Cu had the highest variability.

Correlations between the same elements in the different components of the ecosystem were significant in most cases (Table 2), with Ca and Mn having the strongest relationship. Correlations between the superficial and sub-superficial soil (soil subsystem) tended to be higher than correlations among leaves, leaf fall and litter (tree subsystem). The elements K, Cu and Zn showed negative relationships between the plant and soil components.

Patterns of covariation in the ecosystem

Multiple co-inertia analysis was implemented for leaves, leaf fall, litter, superficial soil and sub-superficial soil. The analysis created individual ordination axes for each ecosystem component, which showed the covariation of chemical elements within each component. The analysis also created common

Table 2. Correlation of the chemical elements between the five ecosystem components.

		LF	LI	TOP	SUB		LF	LI	TOP	SUB
	N					Ca				
LV		**0.52*****	0.09	0.09	0.03		**0.76*****	**0.72*****	**0.43****	0.18
LF			0.27	**0.33***	**0.33***			**0.81*****	**0.57*****	**0.39****
LI				**0.32***	**0.38****				**0.74*****	**0.70*****
TOP					**0.77*****					**0.73*****
	K					Mg				
LV		**0.35****	0.17	**-0.34****	**-0.32***		0.17	**0.58*****	0.27	0.25
LF			**-0.32***	**-0.49*****	**-0.31***			0.26	**-0.40****	**-0.43****
LI				**0.40****	-0.01				**0.42****	**0.31***
TOP					**0.61*****					**0.84*****
	S					P				
LV		0.18	**0.45****	**0.38****	**0.29***		**0.51*****	**0.31***	0.22	0.25
LF			0.25	**0.26***	-0.02			0.14	0.15	0.23
LI				**0.61*****	**0.29***				**0.58*****	**0.38****
TOP					**0.48*****					**0.71*****
	Mn					Zn				
LV		**0.69*****	**0.62*****	**0.37****	0.27		0.27	0.23	0.19	-0.08
LF			**0.85*****	**0.49****	**0.44****			**0.41****	**-0.44****	**-0.62*****
LI				**0.63*****	**0.60*****				0.16	0.04
TOP					**0.92*****					**0.82*****
	Cu									
LV		**0.63*****	0.07	**-0.59*****	**-0.57*****					
LF			**0.30****	**-0.61*****	**-0.62*****					
LI				-0.24	-0.16					
TOP					**0.78*****					

Significant correlations (<0.05) are in bold (***p<0.001; **p<0.01; * p<0.05)
LV (Leaves), LF (Leaf fall), LI (Litter), TOP (Superficial soil), SUB (Subsuperficial soil)

synthetic axes that revealed the structure of the whole dataset and showed how the patterns of variation of each component (individual axes) were related.

All individual ordinations showed a similar trend of nutrient enrichment. In every component of the tree-soil system (leaves, leaf fall, litter, superficial soil and sub-superficial soil), all chemical variables were positively related to the first axis for which most of the elements showed high loadings (Table 3). These results confirmed what we had previously observed with the bivariate correlations: chemical elements covaried in the forest ecosystem along a multivariate gradient of nutrient enrichment that was retained in the first extracted axis of the individual ordinations.

MCoA arranged the axes extracted from each ecosystem component (individual ordination), revealing the relationships and structure of the whole dataset. The first individual axis of every component was strongly related to other first axes and with the common synthetic axis extracted from the whole dataset ordination (high factor loadings > 0.95; Table 4, Fig. 3). This highlighted the strong match between the multivariate structures of the individual datasets

Table 3. Loading of the variables onto the individual ordination axes of each ecosystem component. (LV = leaves, LF = leaf fall, LI = litter, TOP = superficial soil and SUB = sub-superficial soil).

	LV.1	LV.2	LF.1	LF.2	LI.1	LI.2
N	0.36	-0.73	0.73	-0.27	0.60	0.06
Ca	0.93	0.14	0.99	0.24	0.95	0.31
K	0.66	-0.37	0.14	-0.26	0.70	-0.21
Mg	0.48	-0.15	0.83	-0.16	0.88	-0.24
S	0.87	0.27	0.97	0.05	0.85	-0.03
P	0.84	0.36	0.96	0.57	0.78	0.51
Mn	0.41	-0.16	0.23	-0.22	0.46	-0.17
Zn	0.65	0.08	0.48	-0.44	0.53	-0.29
Cu	0.18	-0.46	0.55	-0.49	0.14	-0.88

	TOP.1	TOP.2	SUB.1	SUB.2
pH	0.71	-0.11	0.64	0.03
C	0.79	-0.44	0.82	-0.60
N	0.91	-0.38	0.90	-0.41
N-NH4^{+}	0.31	-0.51	0.45	-0.13
P-PO$_4^{3-}$	0.21	-0.66	0.42	-0.47
CEC	0.80	0.12	0.64	0.46
CEC.Ca	0.83	-0.06	0.73	0.28
CEC.K	0.72	0.07	0.28	0.10
CEC.Mg	0.53	0.41	0.55	0.62
AEDT.S	0.21	0.09	0.50	-0.13
AEDT.Mn	0.78	-0.15	0.75	-0.20
AEDT.Zn	0.47	0.04	0.40	0.01
AEDT.Cu	0.68	0.42	0.45	0.38
Ca	0.90	-0.24	0.73	0.19
K	0.60	0.65	0.29	0.80
Mg	0.72	0.55	0.42	0.76
S	0.78	-0.07	0.76	0.28
P	0.84	0.09	0.70	0.19
Mn	0.76	-0.05	0.85	-0.31
Zn	0.85	0.10	0.61	0.29
Cu	0.85	0.30	0.54	0.05

CEC: Cations extracted with amonium acetate
AEDT: Micronutrients extracted with acid ethylenediaminetetraacetic

and showed that the pattern of nutrient enrichment was consistent across all ecosystem components. Therefore, those trees with leaves with a higher nutrient concentration also presented higher nutrient concentration in their leaf fall and litter and occupied richer soils.

The first axis of each component (individual ordination) and the first common synthetic axis separated the samples of the two studied oak species, regardless of the forest site (Table 5). The average scores of *Q. canariensis* were significantly higher than those of *Q. suber* in all main axes. This result demonstrates that all leaves, leaf fall, litter and soil of *Q. canariensis* tended to have higher nutrient concentrations.

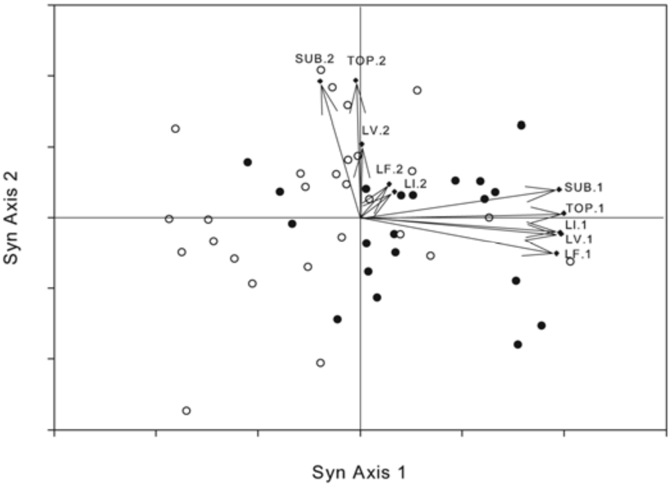

Figure 3. Representation of the projected individual ordination axes (see abbreviations in Table 4) and the tree scores on the synthetic common axes. Filled symbols represent *Q. canariensis* trees, and hollow symbols represent *Q. suber* trees.

Table 4. Loading of the individual ordination axes (LV = leaves, LF = leaf fall, LI = litter, TOP = superficial soil and SUB = sub-superficial soil) onto the common synthetic axes (SYN).

	SYN.1	SYN.2
LV.1	0.98	-0.12
LV.2	0.01	0.52
LF.1	0.96	-0.25
LF.2	0.14	0.24
LI.1	0.98	-0.11
LI.2	0.17	0.18
TOP.1	1.00	0.03
TOP.2	-0.02	0.97
SUB.1	0.97	0.20
SUB.2	-0.20	0.96

Table 5. ANOVA for the oak species' effect on the first multivariate individual ordination axis extracted for each ecosystem component. Only data from mixed forest sites (La Sauceda and Tiradero) were included (n = 40).

	F	p
LV.1	94.721	0.000
LF.1	18.474	0.000
LI.1	28.406	0.000
TOP.1	8.524	0.006
SUB.1	6.007	0.019
SYN.1	18.798	0.000

(LV = leaves, LF = leaf fall, LI = litter, TOP = superficial soil, SUB = sub-superficial soil and common synthetic axes =SYN).

Tree-soil positive feedback model

Multiple co-inertia analysis showed that all materials shared a common multivariate pattern of nutrient enrichment based on which chemical element concentrations in the plant and in the soil were strongly related. In addition, the distribution of the two species along the multivariate axes indicated that both oaks differed in the nutrient richness of their associated soils and plant tissues. This relationship suggests the existence of a positive feedback mechanism between trees and soil in which trees of different oak species, via differences in their nutrient return, distinctly modify soil conditions. This, in turn, would also influence seedling establishment and ultimately affect species distribution.

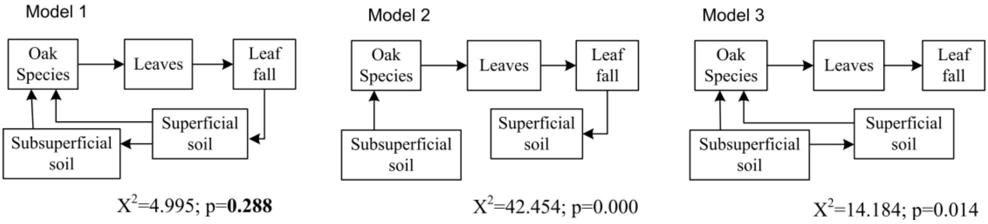

Figure 4. Examples of the alternative causal models tested using the d-sep method. Model 1 = tree-soil positive feedback effect; Model 2 = species affect the superficial soil via nutrient return, but there is no feedback; Model 3 = only soil affects species distribution, there is no feedback and no species effect on soil.

To unveil the causal relationship that linked the tree and soil subsystems and determine whether our hypothesized feedback model fits the empirical observations, we use d-path analysis to test several alternative models (Fig. 4; not all alternative models are shown). The main underlying hypotheses were: 1) oak species affect soil conditions via nutrient return, and this generates a positive feedback effect; 2) sub-superficial soil influences species distribution, and in turn, species modify superficial soil conditions via nutrient return, but there are no feedback effects; and 3) soil affects species distribution, but trees have no effect on soil conditions. We used the first extracted axis for each ecosystem component in the individual ordination as latent variables in the d-sep analysis. Leaf fall was used as a proxy of nutrient return because both leaf fall and leaf litter were equally related to leaves ($r_s = 0.66$) and this distorted the order of the causal chain (Shipley, 2000). The analysis showed that the structure of variance and covariance of the interrelated variables from the alternative models based on the positive feedback hypothesis fit the empirical observations (i.e., Model 1; $\chi^2 = 4.925$, $p = 0.177$). Models based on the other two alternative hypotheses were rejected ($p < 0.05$). These results demonstrated that the tree-soil positive feedback model was the most feasible model.

DISCUSSION

Variability of tree and soil chemical composition

We examined the variability and relationships of the chemical compositions of five components in the forest ecosystem. We determined that there is reciprocal control of their elemental composition resulting from the exchange of chemical elements.

Both oak species, *Q. suber* and *Q. canariensis,* showed similar patterns of variability in the concentration of chemical elements in their tissues and surrounding soil. In contrast, we found an important difference between the variability of the chemical properties measured in the tree system (leaves, leaf fall and litter; CVs of approximately 20-30%) and those quantified in the soil subsystems (CVs of 55-75%). Similarly, Ladanai et al. (2010) found no differences between the variability in the chemical composition of the needles and in the soil humus of two conifer species in Sweden but observed a large difference in the variability between the two ecosystem components (20% for the needles, 60% for the humus). They explained these differences by stating that leaves are part of one organism with homeostatic control on its internal chemical balance, while soil chemical composition is the result of the activity of many organisms.

Despite the differences in the ranges of CVs, we found a strong coupling between the variability patterns in the different ecosystem components. Those elements with great variance of concentration in the leaves tended to also have great variance in the others components. A similar, but weaker, coupled relationship between tree leaves and soil has been found in boreal forests (Merila and Derome, 2008; Ladanai et al., 2010). This suggests that there is a general gradient between chemical elements with low variability (S, P, and N) and elements with high variability (Ca, Mn and K) in the forest ecosystem.

Our results confirmed the initial hypothesis that the variability of elements will differ depending on their degree of biological control, chemical bonding properties and sources. Those elements showing the lowest variability (N and S) are both controlled by biological redox reactions. Together with P, they form organic molecules with covalent bonds. Oak trees have homeostatic regulation of their internal physiologically active nutrient composition (specifically that of N, S and P), which determines their low variability. In contrast, non-limiting elements are usually absorbed in concentrations exceeding the physiological needs, and their uptake is poorly regulated. This can lead to highly variable concentrations of these elements in plant tissues, which can reflect the soil concentration patterns (Knecht and Goransson, 2004). This would be expected to be true for metal macronutrients that originate from mineral weathering and have varying bonding properties. For example, K occurs as an electrolyte in cell sap or as a positive counter-ion to negative surfaces (proteins), and Ca often occurs bound in a complex form in organic molecules (cell wall, chlorophyll) or is deposited as insoluble salts (oxalates) (Ladanai et al., 2010). In these forest systems, the concentrations of micronutrients (Mn, Cu and Zn) in the living leaves were not more variable than those of other measured elements despite the higher variability in soil (Fig. 1). The high variability of these elements often reported in studies at a global or regional scale (Wood et al., 2006; Watanabe et al., 2007) is a multi-species pattern reflecting the presence of hyperaccumulator species in the forest community. Oak species are known leaf accumulators of Mn (Madejón et al., 2006). For example, *Q. canariensis* leaves in the study area reached up to 1200 mg kg^{-1} of Mn, but they are relatively well regulated (CV of approximately 30%) considering the very high variability of soil Mn (CV above 100%, Fig. 1).

There was a strong correlation of the concentration of chemical elements between the superficial and sub-superficial soil (significantly positive for the nine elements). This correlation was weaker among the tree components (strong for Ca and Mn, but with 10 non-significant r values among the 21 other elements). In soil, mineral elements are chemically bound to organic matter, and their balance is largely controlled by purely chemical exchange reactions. This results in a stronger covariation of elements in this subsystem. In the tree system, there is a differential control on essential and non-essential elements (Knecht and Goransson, 2004; Ladanai et al., 2010).

The significant correlation found for some chemical elements (Ca, Mn, N and P) between different components of the ecosystem suggested that nutrient concentration and stoichiometry for structural and organically bound elements is transferred across ecosystem components (Perakis et al., 2006; Merila and Derome, 2008). However, other elements (i.e., K, Cu and Zn) showed a lack of coupling between vegetation and soil chemical compositions (see also Wood et al., 2006; Ladanai et al., 2010). Potassium is not a structural component of plant tissue. Thus, dead tissue (litter) would not hold K against leaching and decomposition. In fact, Laskowski (1995) found that K release does not correlate with microorganism activities but is rather correlated with leaching. Because of their morphological traits, deciduous leaves with a higher K content would be subjected to a more intense leaching of this labile element and would render litter and soil with lower concentrations of K. In contrast, the thick cuticles of evergreen leaves would impede K removal and would thus favor the retention of this element in the litter and organic soil molecules. Copper and Zn between the plant and the soil subsystems were negatively related. MCoA analysis showed high loadings for these two elements on the soil fertility gradient (≈ 0.70), but lower loadings for the plant ordination axis compared to other chemical elements (≈ 0.29 for Zn, ≈ 0.50 for Cu). Several mechanisms can result in a lack of a relationship between element concentrations in leaves (plant system) and soil. For example, protective mechanisms in the root cell environment might impede excessive uptake of potentially toxic elements (Hall, 2002). Additionally, elements can be retained in the roots and not transferred to shoots (Domínguez et al., 2009), or plants can experience antagonistic interactions of other elements, inhibiting uptake (Madejón et al., 2006).

Multivariate patterns of nutrient covariation

Results from the multiple co-inertia analysis showed the existence of a common enrichment pattern in each ecosystem component. Multivariate ordination techniques are powerful tools for describing meaningful patterns without focusing on particular variables (Jongman et al., 1995). However, multi-table ordination techniques such as MCoA are rarely used in ecology, despite their ability to deal with spatial and temporal stabilities of environmental properties and community compositions (Dray et al., 2003; Bady et al., 2004). The investigation of ecosystem dynamics usually requires the simultaneous analysis of several multivariable datasets. Thus, MCoA is a very interesting tool to explore general patterns in ecosystems and implementation and interpretation are easy for users familiarized with ordination techniques such as principal component analysis (Daufresne et al., 2007).

Both the bivariate correlations and the independent multivariable ordination of the ecosystem components showed that most chemical elements covaried along a main enrichment gradient. This result indicated that in all the tree components (leaves, leaf fall and litter) the abundance of organically bound elements (N, P and S) was associated with high levels of other macronutrients (Ca, Mg and K) and micronutrients (Zn, Cu and Mn). Previous studies had only focused on the concentrations of elements in leaves and had documented specific correlations. Garten (1976) reported correlations between N, P, Mg and Ca across a variety of plant species. Alonso and Herrera (2001, 2003) observed a high positive correlation between macronutrients (N, P, K, Ca and Mg) that were responsible for the structure of covariation between concentrations of the leaf nutrients across individuals of *Prunus mahaleb*.

Nutrients in plants are related to the intrinsic chemical properties and biochemical functionality in cell metabolism. Calcium, Mg and Mn are structural elements found in cell walls and chloroplasts. Nitrogen, P and S are incorporated in macromolecules. Potassium, Mg and Mn activate some of the enzymes involved in plant cell metabolism. Copper is essential to electrotransport enzymes, and Zn is necessary to make indolacetic acid, a growth hormone in plants (Salisbury and Ross, 1969). Thus, relationships between concentrations of elements may not vary randomly, and they may be a consequence of similarity in physiological function or nutrient uptake and accumulation (Garten, 1976, 1978; Sterner and Elser, 2009). Recent advances in the study of the elemental composition of leaves (ionomics) and in the study of the plant genome are contributing to our understanding of the physiological and biochemical mechanisms that determine plant genetic and environmental responses (Baxter et al., 2008).

A common pattern of fertility was found for the superficial and sub-superficial soil. Higher concentrations and availability of mineral nutrients was related to higher soil C content and soil pH. Most nutrients in forest soils are bound to soil organic matter. The abundance of these nutrients can be directly related to soil C content in acidic soils. Nitrogen and S are constituents of soil organic molecules, whereas P can be weathered from the bedrock, although organic P is the major source of labile P. The availability of micronutrients (Mn, Cu and Zn) is often controlled by soil organic matter turnover, although the ultimate source of these elements is mineral weathering (Fisher and Binkley, 2000). At the same time, soil acidity influences mineral solubility, mineral weathering and the distribution of cations in the exchange complex. Therefore, soil acidity controls inorganic nutrient availability. Calcium in forest soils is a key element for soil pH and is strongly related with forest floor biogeochemical dynamics. Thus, increased soil Ca can raise the exchangeable base saturation, mitigate soil acidity and influence nutrient availability, subsequently affecting decomposition rates and soil community composition (Reich et al., 2005; Hobbie et al., 2006; Aponte et al., 2010).

The multiple co-inertia analysis showed that in addition to the main gradient of nutrient enrichment found in each of the five ecosystem compartments, there was also a multivariate pattern of nutrient covariation that remained consistent across compartments. Nutrient cycling in ecosystems involves the storage and transfer of elements. The chemical elements studied here covaried in leaves, leaf fall, litter, superficial soil and sub-superficial soil despite the distinct variability found for mineral and organically bound elements. This suggests that the chemical elements and their stoichiometric relationships are transferred across the ecosystem components through the organic matter cycle (Cornwell et al., 2008).

A remarkable result obtained from the MCoA was the significant separation of the two oaks species in the multivariate space defined by the chemical composition of each (individual ordination) and all (common synthetic ordination) ecosystem components. This result suggested a separation in their biogeochemical niches, which would be the combined result of genotypic interactions (absorption and accumulation capacity and functional stoichiometry) and environmental interactions (Garten, 1978; Peñuelas et al., 2008). The deciduous *Q. canariensis* had higher multivariate scores than the evergreen *Q. suber*, reflecting its higher nutrient concentrations in leaves, litter and soil. These results support our hypothesis and are in accordance with the previously reported differences between deciduous and evergreen species in terms of resource acquisition and cycling strategies (Hobbie, 1992; Aerts, 1995). Evergreen species usually occur at acidic, nutrient-poor sites, whereas deciduous species commonly dominate in fertile soils with higher nutrient content and soil pH (Goldberg, 1982).

In general, deciduous trees are characterized by a higher specific leaf area and relative growth rate in productive areas with no limiting resources. They have greater efficiency in acquiring and photosynthesizing light, allowing them to outcompete evergreens in favorable conditions (Cornelissen et al., 1996; Antúnez et al., 2001). In comparison, evergreen trees generally show slower relative growth rates as a consequence of the lower specific leaf area and photosynthetic rates of their longer-lived leaves (Cornelissen et al., 1999; Castro-Díez et al., 2000). However, because of their low growth rate, they have a lower nutrient demand and nutrient loss that allows them to persist in nutrient-poor soils (Aerts, 1995). The trade off between growth and defense is reflected in the different chemical composition of evergreen and deciduous leaves, leaf fall and litter (Villar et al., 2006). Fast-growing deciduous species commonly have higher levels of minerals, organic acids, proteins and lipids, whereas leaves of the slow-growing evergreen species have higher concentrations of soluble phenolics and lignin, as well as higher carbon:nutrient element ratios (Reich et al., 1992; Cornelissen et al., 1997). Therefore, the interspecific differences in leaf traits (functional morphology and composition) have ecological consequences for the biogeochemical niches of *Q. canariensis* and *Q. suber* that favor their coexistence and also cause the species to impose different footprints on the ecosystem.

Tree-soil positive feedback effects in Mediterranean forests

In this study, we have demonstrated the feasibility of a tree-soil positive feedback effect by implementing a causal modeling approach and testing several alternative competing models. In this context, feedback effects can be modeled as cyclic causal relationships that must be understood as a fixed image of a system dynamic occurring in time (Shipley, 2000). Feedback effects must be interpreted cautiously because it is often difficult to discern the origin of the cyclic causal relationship. Our analyses showed that differences between the two species were highly significant ($p< 0.01$) in the plant components as well as in the surface soil. Differences were attenuated in the sub-superficial soil ($p< 0.05$). These results suggest that tree species modify soil conditions by a top-down cascade. This finding supports our hypothesized feedback model. The investigation of tree-soil relationships has commonly been based on unidirectional studies that have mainly focused on tree species' effects on soil properties, and the feedback effect on the plant population has been neglected (Finzi et al., 1998; Laurent et al., 2002; Eviner et al., 2006). To confirm the existence of a feedback effect, the modifications created by a tree species must have an impact (positive or negative) on the performance and fitness of individuals of the same species (Ehrenfeld et al., 2005).

Our results showed that *Q. canariensis* trees produced leaves with a higher nutrient content than the co-occurring *Q. suber* trees. These differences in the chemical compositions of leaves were transferred to the species' leaf fall and litter, leading to a differential nutrient return that distinctly modified soil conditions underneath each oak species. Thus, *Q. canariensis* trees increased the soil fertility levels through a higher nutrient input. In addition, as a consequence of an elevated input of Ca cations, they might have lowered soil acidity and increased nutrient availability both in the surface and the subsurface soil. According to our initial hypothesis, the more fertile conditions generated by the deciduous oak could have positive consequences for the establishment of individuals of the same species if the occurrence of the species is associated with these nutrient-enriched environments. In contrast, *Q. suber* has long-lived leaves with a lower nutrient content and consequently a lower nutrient return. This species generated nutrient-poor environments, where their less nutrient-demanding seedlings could have higher fitness. This positive tree-soil feedback model indicated that the two oak species each have a distinct effect on nutrient cycling that can differentially benefit their recruits (nutrient-demanding versus scarcity-resistant) and ultimately contribute to their coexistence. Previous studies in Mediterranean oak forests have shown that winter deciduous trees and evergreen oaks have a different regeneration niche (Maltez-Mouro et al., 2005). Winter deciduous trees have a higher recruitment at microsites closer to conspecific adults and with a high percentage of canopy cover and litter mass, whereas evergreen oak seedling performance is enhanced at microsites with lower litter mass and canopy cover (Maltez-Mouro et al., 2009). A recent study conducted in the same study area demonstrated that *Q. canariensis* seedlings grew faster than those of *Q. suber* beneath the canopy of a con-specific adult (F=10.31, p< 0.009; data taken from Pérez-Ramos et al., 2010). The strong positive relationship found between soil fertility, litter mass and percentage of cover supports our hypothesis that soil fertility might influence species distribution (García et al., 2006).

Evidence that plants can create positive feedback effects through their influence on nutrient cycling has been previously reported for temperate and boreal forests (Hobbie, 1992; Aerts, 1995). Low nutrient content and structural traits associated with the long-lived leaves of evergreen trees (lower specific leaf area, lower palatability, higher tensile strength) render poor-quality litter that decomposes slowly (Gallardo and Merino, 1993). These features minimize nutrient loss and keep a low rate of nutrient mineralization that might help them to maintain their position in infertile sites (Cornelissen et al., 1999). Additionally, this does not allow potentially fast-growing deciduous species to outcompete them (Cornelissen et al., 1999). In contrast, deciduous trees produce short-lived leaves with a higher nutrient concentration and specific leaf area that yield high decomposition rates, further increasing soil fertility and enhancing the competitive ability of their own seedlings (Hobbie, 1992; Antúnez et al., 2001).

Plant-soil feedback effects represent the net effect of a variety of potentially negative and positive interactions within the soil subsystem (Wardle, 2002). Negative feedback effects originated by the accumulation of soil pathogens are common in early successional communities, whereas positive (or neutral) feedback effects are expected for shrubs and trees in late successional communities (Kardol et al., 2006; Kulmatiski et al., 2008). Associations with ectomycorrhizal fungi are affected by the tree-modified rhizosphere environment (Aponte et al., 2010) and may contribute to the positive feedback effects. Additionally, the protective mycorrhizal sheaths around the rootlets can inhibit pathogen infection (Duchesne et al., 1989) and reinforce the positive feedback.

The ability of species to control nutrient cycling and enhance their own fitness has important evolutionary implications (Berendse, 1994; van Breemen and Finzi, 1998; Post and Palkovacs, 2009). In our study, evergreen and deciduous oaks generated a positive feedback effect that could confer stability in species interactions and maintain the structure and diversity associated with both coexisting species (Ojeda et al., 2000; Aponte et al., 2010). Recent studies have demonstrated that climate change affects nutrient availability and accumulation in plants (Sardans and Peñuelas, 2004, 2007), which implies changes in the biogeochemical niche of the species, and affects their relationships with nutrient cycling (Peñuelas et al., 2008). These alterations in tree-soil interactions may change interspecific competitive relationships and subsequently modify the species composition of vegetation and soil biota.

Conclusions and future directions for tree-soil feedback effect research

To study the tree-soil feedback effects of two coexisting oak species in the Mediterranean forest, we investigated the relationships between the chemical compositions of five different components of the ecosystem. Chemical elements varied in their concentration depending on their biogeochemical cycles, but this variability was consistent across all ecosystem components. Multiple co-inertia analysis revealed that there was a common multivariate pattern of nutrient enrichment in the ecosystem that separated the biogeochemical niches of the two oak species. The deciduous *Q. canariensis* occupied more fertile environments and had more enriched tissues than the evergreen *Q. suber*. A theoretical positive feedback model was validated using path analysis. This model suggested that oak species, through their differential nutrient return, distinctly modify soil properties that can affect seedling recruitment and, on a long-term scale, species distribution. This feedback dynamic might be key for the stability of the forest ecosystem and the coexistence of the two oak species.

Theoretical models based on observational data (as was conducted in this study) are an important step in the study of tree-soil interactions. However, further investigations are needed to fully understand the role of feedback effects in forest dynamics. Our knowledge of the complexity of the tree-soil interactions will be enhanced by demographic studies that investigate the mechanisms of the feedback effects of the species through the interactions of seedlings with soil microorganisms, experimental approaches that could determine the rate and persistence of these feedbacks and spatially explicit studies that allow modeling of complex situations in mixed forests where species' footprints on soil properties overlap.

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APPENDICES

Appendix 1. Descriptive of the chemical composition of the leaves, leaf fall and litter. Median and coeficient of variation (%).

		Leaves				Leaf fall				Litter			
		Q. canariensis		*Q. suber*		*Q. canariensis*		*Q. suber*		*Q. canariensis*		*Q. suber*	
		Median	CV	Median	CV	Median	CV	Median	CV	Median	CV	Median	CV
N	%	1.59	15.0	1.40	12.4	0.92	18.9	0.85	13.6	1.06	12.7	1.09	15.1
K	%	0.95	23.1	0.55	35.2	0.19	33.1	0.21	33.4	0.15	16.5	0.12	16.3
Ca	%	0.76	32.4	0.41	19.2	1.40	19.5	0.86	15.7	1.60	32.6	0.96	21.9
Mg	%	0.15	27.3	0.13	20.4	0.16	24.9	0.14	15.2	0.17	19.8	0.14	12.5
S	%	0.13	9.2	0.11	5.6	0.12	24.8	0.11	20.3	0.13	8.20	0.12	10.6
P	%	0.09	14.9	0.07	13.9	0.05	27.5	0.04	29.3	0.05	17.9	0.05	15.1
Mn	mg kg^{-1}	749	28.7	605	30.5	1020	25.2	792.5	27.5	1025	20.1	814	29.6
Zn	mg kg^{-1}	19.0	20.7	16.8	19.0	25.2	55.3	19.5	35.4	36.9	24.0	27.9	23.0
Cu	mg kg^{-1}	3.90	24.9	3.95	22.0	6.93	40.2	5.60	32.5	7.57	19.8	7.76	16.7

Appendix 2. Descriptive of the chemical properties of the superficial and sub-superficial soil. Median and coeficient of variation (%).

		Superficial soil				Sub-superficial soil			
		Q. canariensis		*Q. suber*		*Q. canariensis*		*Q. suber*	
		Median	CV	Median	CV	Median	CV	Median	CV
pH		4.82	10.1	4.26	9.9	4.17	15.3	3.89	11.2
N-NH_4^+	mg kg^{-1}	6.65	94.6	2.64	119.8	3.34	92.2	5.36	75.9
P-PO_4^{3-}	mg kg^{-1}	3.50	42.3	2.21	73.4	1.38	100.4	1.23	74.6
CEC	mg kg^{-1}	24.5	39.5	22.7	38.9	15.9	51.9	16.5	37.0
CEC.Ca	mg kg^{-1}	1107	61.5	608	77.3	455	131.5	310	100.7
CEC.K	mg kg^{-1}	89	61.7	109	81.2	154	38.0	199	62.9
CEC.Mg	mg kg^{-1}	115	27.8	102	34.9	177	169.0	195	181.4
AEDT.S	mg kg^{-1}	4.03	45.8	3.91	27.3	37.5	93.6	80.0	69.5
AEDT.Cu	mg kg^{-1}	1.08	111.1	0.89	85.8	1.26	114.2	1.40	61.5
AEDT.Mn	mg kg^{-1}	224	49.2	101	72.4	81.2	120.1	32.6	138.4
AEDT.Zn	mg kg^{-1}	4.07	132.2	3.37	45.6	1.73	59.0	2.15	57.6
N	%	0.26	31.2	0.20	33.2	0.12	37.0	0.11	25.4
Ca	mg kg^{-1}	2581	71.3	993	81.5	700	117.5	328	99.7
K	mg kg^{-1}	1935	74.1	1990	59.7	2535	67.5	3255	44.9
Mg	mg kg^{-1}	1819	68.3	1683	58.0	2485	66.3	2678	42.4
S	mg kg^{-1}	246	19.8	216	20.5	151	24.6	149	21.6
P	mg kg^{-1}	258	29.7	216	32.4	211	27.9	203	29.2
Mn	mg kg^{-1}	1343	59.5	543	103.0	662	85.0	207	140.3
Cu	mg kg^{-1}	8.50	87.5	5.97	84.7	7.25	113.8	5.63	104.9
Zn	mg kg^{-1}	35.8	35.8	27.1	44.1	31.9	61.0	17.8	65.6

CEC: Cations extracted with ammonium acetate

AEDT: Micronutrients extracted with acid ethylenediaminetetraacetic

Appendix 3. Correlation of chemical elements within the five ecosystem components considered in this study. Significant correlation ($p<0.05$) are in bold (***$p<0.001$; **$p<0.01$; * $p<0.05$)

	Ca	K	Mg	S	P	Mn	Cu	Zn	Ca	K	Mg	S	P	Mn	Cu	Zn
	Leaves								Superficial soil							
N	0.23	**0.52*****	-0.13	**0.40*****	**0.46*****	0.12	**0.48*****	**0.39****	**0.68*****	**0.46*****	**0.49*****	**0.68*****	**0.78*****	**0.61*****	**0.57*****	**0.65*****
Ca		**0.63*****	**0.51*****	**0.77*****	**0.57*****	**0.58*****	-0.04	**0.41****		**0.70*****	**0.79*****	**0.42*****	**0.68*****	**0.77*****	**0.85*****	**0.79*****
K			0..17	**0.46*****	**0.63*****	0.24	**0.42*****	**0.33***			**0.97*****	**0.33***	**0.75*****	**0.71*****	**0.92*****	**0.75*****
Mg				**0.44*****	0.24	**0.34****	-0.19	0.04				**0.31***	**0.75*****	**0.78*****	**0.96*****	**0.81*****
S					**0.59*****	**0.33***	-0.08	**0.42****					**0.67*****	**0.38****	**0.35****	**0.45*****
P						**0.34****	0.19	**0.41****						**0.79*****	**0.78*****	**0.84*****
Mn							-0.03	0.24							**0.83*****	**0.90*****
Cu								0.25								**0.86*****
Zn																
	Leaf fall								Sub-superficial soil							
N	**0.35****	**0.45*****	**0.61*****	**0.70*****	**0.72*****	**0.33***	**0.72*****	**0.54*****	0.23	0.11	0.16	**0.61*****	**0.68*****	**0.50*****	0.23	**0.36****
Ca		-0.07	**0.44****	0.22	**0.39****	**0.52*****	0.13	0.03		**0.43****	**0.72*****	**0.49*****	**0.44*****	**0.73*****	**0.85*****	**0.82*****
K			**0.51*****	**0.68*****	**0.65*****	0.00	**0.68*****	**0.62*****			**0.85*****	**0.46*****	**0.56*****	0.03	**0.52*****	**0.34****
Mg				**0.70*****	**0.68*****	0.20	**0.61*****	**0.58*****				**0.51*****	**0.55*****	**0.37****	**0.81*****	**0.65*****
S					**0.83*****	0.07	**0.91*****	**0.88*****					**0.86*****	**0.59*****	**0.41****	**0.61*****
P						0.25	**0.76*****	**0.62*****						**0.41****	**0.43****	**0.53*****
Mn							0.19	-0.04							**0.67*****	**0.79*****
Cu								**0.84*****								**0.85*****
Zn																
	Litter															
N	-0.09	-0.13	0.22	**0.59*****	**0.46*****	**-0.29***	**0.42****	-0.14								
Ca		**0.78*****	**0.73*****	**0.58*****	**0.50*****	**0.55*****	0.02	**0.29***								
K			**0.67*****	**0.40****	**0.43****	**0.48*****	0.04	0.12								
Mg				**0.63*****	**0.57*****	**0.38****	0.15	0.22								
S					**0.75*****	0.18	**0.39****	**0.40****								
P						0.19	**0.30***	0.21								
Mn							0.14	**0.34***								
Cu								0.24								
Zn																

Capítulo 5.

Factores que afectan a la descomposición de la hojarasca y la incorporación de los minerales al suelo

Este capítulo reproduce el siguiente manuscrito:

Aponte, C, García, L.V., Marañón, T. *Leaf litter decomposition in a mixed oak forest: factors controlling decay rates and nutrient dynamics* (en preparación).

Capítulo 5. **Factores que afectan a la descomposición de la hojarasca y la incorporación de los minerales al suelo**

RESUMEN

En este trabajo se investigó la influencia directa –a través de la calidad del desfronde- e indirecta - mediante sus efectos sobre las condiciones ambientales - de dos especies de quercíneas coexistentes, la perennifolia *Q. suber* y la marcescente *Q. canariensis*, en la dinámica de descomposición de la hojarasca. También se estudiaron los efectos no aditivos de la mezcla de especies y se evaluó la teoría de la "ventaja de jugar en casa" (*home field advantage*) que sostiene que la hojarasca de una especie se descompone más rápidamente bajo el dosel de la especie que la produce. El proceso de descomposición se investigó mediante el uso de bolsas de hojarasca que se incubaron bajo cobertura de *Q. canariensis* y *Q. suber*, debajo de matorral y en claros del bosque con vegetación herbácea y se recolectaron cada seis meses durante dos años. Se determinaron la pérdida de biomasa y la concentración de nutrientes (Ca, Mg, K, P, S, Mn, Cu, Zn) de la hojarasca incubada así como 17 parámetros químicos, físicos y biológicos del suelo.

La pérdida de biomasa se ajustó a un modelo de descomposición asintótico con dos etapas diferenciadas. Durante la primera etapa de la descomposición, la hojarasca de *Q. canariensis* se descompuso a un ritmo superior a la de *Q. suber*. En la segunda etapa de la descomposición las condiciones ambientales bajo *Q. canariensis* promovieron la descomposición de la hojarasca de ambas especies dando lugar a una mayor pérdida de biomasa. La concentración de calcio en la hojarasca y en el suelo emergió como el mejor predictor de los parámetros de la descomposición. La dinámica de liberación de los nutrientes de la hojarasca fue muy variable dependiendo del elemento.

Los resultados indican que en estos bosques mediterráneos, las especies de *Quercus* influyen en la dinámica de la descomposición a través de la calidad de de su desfronde y de las diferentes condiciones del suelo que generan. Los cambios en el contenido de calcio de la hojarasca y el suelo explican en un grado significativo las diferencias observadas en la dinámica de descomposición.

Leaf litter decomposition in a mixed oak forest: factors controlling decay rates and nutrient dynamics

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Abstract

Tree species may have both a direct influence on decomposition process, through the quality of their leaf fall, and an indirect effect via the species-specific condition they generate in their environment. We studied the direct and indirect influences of species identity on the litter decomposition dynamics in Mediterranean forests. We also evaluated the occurrence of two phenomena associated to decomposition in mixed forest: the non additive response of species litter mixture and the "home field advantage" effect.

Litterbags with litter of the winter deciduous Quercus canariensis, of the evergreen Q. suber and mixed litter were incubated beneath distinct plant covers in two forest of south Spain during two years. Litter mass loss, litter nutrient (Ca, Mg, K, P, S, Mn, Cu, Zn) concentration and 17 soil chemical, physical and biological parameters were measured. Additionally, we investigated the forest floor decomposition dynamics by mass balance using empirical observations of annual leaf fall and litter mass.

Litter mass loss was best described by an asymptotic decay model that differentiated two decomposition periods. During the first stage of decomposition, leaf litter of Q. canariensis decayed at a higher rate than Q. suber. In the second stage of decomposition the environment beneath the Q. canariensis promoted all litter types decomposition rendering a higher litter mass loss. Calcium concentration in litter and soil emerged as the best predictors for both litter and forest floor decomposition rates. We observed a non additive response of Q. canariensis litter, which decay was promoted in mixed litterbags whereas we found no evidences to support the "home field advantage" theory. Differences in the initial litter nutrient concentration, biomass decay and element loss rate between tree species determined the absolute element loss and reflected species distinct mineral cycling.

Our results reveal that in the studied Mediterranean forests species identity influence decay dynamics through both their litter quality and the different soil conditions they generate, and thus tree-soil interactions established in these forest significantly affect nutrient cycling and ecosystem functioning.

Keywords: *calcium, home field advantage, limit value, litter decay, litter mixtures, nutrient release, plant–soil interaction, Quercus canariensis, specificity effects.*

INTRODUCTION

Decomposition of plant litter is an essential process for ecosystem functioning, integral to biogeochemical cycles and nutrient availability for plants (Swift et al., 1979; Aber and Melillo, 2001). Leaf litter decomposition occurs at the aboveground-belowground interface thus variables from both environments interact and affect the decomposition process (Lorenz et al., 2000; Gartner and Cardon, 2004; Vivanco and Austin, 2008). The factors influencing decomposition include litter quality, i.e. the physical and chemical properties of plant leaf litter (Gallardo and Merino, 1993; Cornwell et al., 2008); environmental variables such as temperature, humidity, UV radiation and soil fertility (Hobbie, 1996; Sariyildiz and Anderson, 2003; Austin and Vivanco, 2006); and the decomposer community, including bacteria, fungi, arthropods and worms (Wall et al., 2008; Strickland et al., 2009).

Tree species may affect decomposition through both direct and indirect effects (Vivanco and Austin, 2008). Direct effects are exerted through their leaf litter properties such as C:N ratio, lignin and cutin content, tensile strength, Ca concentration and pH (Pérez-Harguindeguy et al., 2000; Cornelissen et al., 2006; Hobbie et al., 2006). Indirect effects are related to the species–specific conditions that trees generate in their abiotic and biotic environment throughout changes in litter and soil fertility, and microclimate that render distinct faunal and microbial communities in the forest floor (Chadwick et al., 1998; Mitchell et al., 2007; Aponte et al., 2010b, c). Few studies have investigated the relative importance of species direct and indirect effect on decomposition in Mediterranean forests whereas there is an increasing number of similar studies in other ecosystems (Hobbie et al., 2006; Vivanco and Austin, 2008). Some of these studies have demonstrated the existence of an affinity effect between species substrate and environment, i.e. litter decomposes faster beneath the plant species from which it is derived (at home) than beneath different plant species (away) (Negrete-Yankelevich et al., 2008; Ayres et al., 2009). This phenomenon, which was referred to as "home-field advantage" could be explained through the adaptation of local soil communities to the litter produced by the plant species above them (Gholz et al., 2000), but it does not always occur (Prescott et al., 2000; Chapman and Koch, 2007).

Individual species effect on decomposition may differ in mixed forest conditions due to positive or negative interactions among litter types (Hättenschwiler and Gasser, 2005; Ball et al., 2008). Decay dynamics of mixed litters can be additive, i.e. species decompose independently thus decay dynamics of single litters can predict decay dynamics of the mixed litters. Alternatively, if a species decomposition depend on the presence of other species, then mixed litters will show a non-additive pattern that can not be predicted from the single litter dynamics (Ball et al., 2008). Potential mechanisms for non-additive patterns would include nutrient transfer between distinct quality litters by diffusion or fungal hyphae, stimulation of microbial processing and alterations in detritivore behaviour (Hättenschwiler et al., 2005; Schimel and Hättenschwiler, 2007; Chapman and Newman, 2010). Both additive and non-additive effects on mass loss and nutrient content have been detected in mixed forest leaf litters (see review in Gartner and Cardon, 2004).

Leaf litter decomposition is widely studied using the litterbag approach (Karberg et al., 2008). This method allows to experimentally quantify the influence of different factors in decomposition rates, but depending on the mesh size it may exclude certain macroinvertebrates from the litterbag, which influences rates estimates (Bradford et al., 2002). To determine whether the factors that affect litter decomposition when litter is contained in litterbags also explain forest floor dynamics when litter is accessible to all soil fauna a forest mass balance approach can be used (Hobbie et al., 2006). This approach determines forest floor removal rates based on the annual input of fresh litter and the accumulated litter mass (Olson, 1963; Schlesinger, 1997). It provides a robust and independent estimate of litter decomposition at the stand level assuming a steady-state stand condition that can complement litterbag results (Karberg et al., 2008).

The dynamics of nutrient release from leaf litter is largely diverse, even for litters of a similar type (Laskowski et al., 1995). The decay patterns of chemical elements seem to be influenced by the nutrient availability to decomposers (Swift et al., 1979; Staaf and Berg, 1982). Limiting nutrients occurring in sub-optimal amounts would be accumulated during the initial stages of litter decomposition, while nutrients exceeding the needs of decomposers could be released from the litter from the very start of decomposition (Laskowski et al., 1995). The analysis of changes in the absolute elements amounts in addition to element concentration in distinct litter types will reflect differences in mineral cycling between tree species.

We aimed to study the leaf litter decomposition process of two co-occurring oak species, the evergreen *Q. suber* and the winter deciduous *Q. canariensis*, and to disentangle the effects that species have on litter decay via their leaf litter quality and their effect on the decomposition environment. Specifically, we pursued four main objectives. First, we aimed to investigate the species characteristic leaf litter chemistry effects on decomposition. We hypothesized that the enriched leaves of *Q. canariensis* will have a higher decomposition rate. Second we analyzed the effect of species litter mixture. We expected that leaf litter mixture would render a synergistic interaction, thus enhancing the decomposition of *Q. suber* litter in mixed conditions. Third, we evaluated how different oak species affects decomposition rates by the differential soil environment they generate underneath. According to the field advantage theory, we hypothesized that each species litter would decompose faster beneath their own species canopy because of a positive substrate-environment interaction. Fourth, we analyzed the relationship between several soil variables potentially influenced by tree species and the decomposition rates and evaluated the extent to which the litter decomposition and forest floor removal rates may be explained by this soil conditions. Fifth, we studied the decomposition dynamics of the leaf litter chemical elements and evaluated the differences in liberation and immobilization of nutrients from the litter of the two oak species.

METHODS

Study area

This study was conducted in the Aljibe Mountains, near the Strait of Gibraltar, south of Spain. The bedrock is dominated by Oligo-Miocene sandstone that originates acidic, nutrient-poor soils (Palexeralfs) frequently interspersed with layers of marl sediments that yield soils richer in clay (Haploxererts; nomenclature follows Soil Survey Staff 2010). The climate is sub-humid Mediterranean type, with a dry and warm summer period of 3-4 months and most rainfall (95%) occurring from October to May. The annual rainfall varies from 701 mm to 1331 mm and temperatures range from 14.6 ºC in the most elevated areas to 18.4 ºC in the coastal zones (Anonymous, 2005). The dominant vegetation is a mixed forest of evergreen cork oak (*Quercus suber* L.) and winter-deciduous Algerian oak (*Q. canariensis* Willd). Both oak species differ in their leaf fall and litter quality: *Q. canariensis* has a higher nutrient content (Ca, K, Mg, S) than *Q. suber*, what induces distinct soil conditions via nutrient return (Aponte et al., 2010a). The arborescent shrubs *Erica arborea* L., *Phillyrea latifolia* L. and *Pistacia lentiscus* L. dominate the understorey (Ojeda et al., 2000). The area has been protected since 1989 as "Los Alcornocales" (meaning "the cork oak forests") Natural Park.

Two structurally different forest sites (40 km apart) were selected within the study area. The site at San Carlos del Tiradero (hereafter called Tiradero) (36º 9’ 46’’ N; 5º 35’ 39’’ W) was located in the south of the Park near the coast at 335–360 m a.s.l. on a NE facing slope. The mean annual rainfall is 964 mm, and the mean annual air temperature is 16.6 ºC, with a minimum of 4.1 ºC. This stand had a high density of trees (769 stems ha^{-1}) with a basal area of 47 m^2 ha^{-1}. The other site, at La Sauceda (hereafter called Sauceda) (36º31’54’’N; 5º34’29’’W) was located inland, in the north of the Park, at 530–560 m a.s.l. on a NW facing slope. It has a mean annual temperature of 15.5ºC, with a minimum of 1.8 ºC and a mean annual rainfall of 1470 mm. The tree density at Sauceda was relatively low with 219 stems ha^{-1} and a basal area of 22 m^2 ha^{-1} (Pérez-Ramos et al., 2008).

Litter decomposition experiment

Freshly senesced leaves of the two oak species were collected from a large forest extension after gently shaking trees at the end of March (for *Q. canariensis*) and June (for *Q. suber*) 2007, during their respective leaf fall period. We prepared 11x11cm litterbags (2 mm fiberglass mess) with 2 g of air-dried leaf litter of the two species and a third type of bags with an equivalent mixture of the two species. We placed the litterbags beneath the canopy of 6 individuals of *Q. suber* and 6 of *Q. canariensis* at the two forest sites. In addition at Sauceda we located litterbags in two other types of microsites: under shrubby cover and in forest gaps with herbaceous vegetation. In total 432 litterbags (3 litter types x 6 microsites x 6 replicates x 4 harvests) were placed in the field in November 2007 and harvested every 6 months for 2 years.

Upon harvest, litter was removed from bags, separated from roots and large soil aggregates, dried (65 ºC, 48h), weighted and analyzed for changes in nutrient content over time. In mixed litterbags, leaves from the two species were carefully separated and hereon treated independently. The proportion of remaining biomass (RB) was calculated by dividing the mass at any harvest date by the initial mass, after correcting for the water content of the initial air-dried samples.

Subsamples of initial leaf litter from each species, as well as the harvested litter samples were grounded and analyzed for total concentration of several nutrients (Ca, K, Mg, P, S, Mn, Cu, Zn) by acid digestion followed by ICP-OES determination. The net change in absolute amounts of chemical element during decomposition of litter was calculated by dividing the element net amount at any harvest date (mg of element per g of remaining litter at that time) by the initial element amount (initial concentration per g of initial litter).

Microsite soil characterization

Several soil inorganic and biologic properties of each microsite were determined (Table 1). Briefly in November 2006, 25 cm deep soil cores were extracted with a cylindrical auger at each microsite. We determined soil pH in a 1:2.5 soil: H_2O solution; the available soil P was estimated using the Bray-Kurtz method; soil NH_4^+ was extracted with KCl (2M) and determined by steam-distillation. The total concentration of several nutrients (Ca, K, Mg, P, S, Mn, Cu, Zn) was determined by acid digestion followed by ICP-OES analysis

Table 1. Description of the incubation microsites where litterbags were located, in the two studied forests (data taken from Aponte et al., 2010a, c and unpublished results). Mean (St. dev).

		Sauceda								Tiradero			
		Q. canariensis		*Q. suber*		Shrub		Herbaceous		*Q. canariensis*		*Q. suber*	
pH		5.85	(0.17)	5.26	(0.38)	6.09	(0.48)	6.33	(0.54)	4.88	(0.24)	4.61	(0.14)
N-NH$_4^+$	mg kg^{-1}	22.3	(11.9)	30.3	(8.0)	33.3	(19.6)	11.2	(3.0)	4.6	(3.4)	2.8	(0.9)
P-PO$_4^{3-}$	mg kg^{-1}	3.31	(0.97)	4.89	(3.56)	2.16	(2.26)	0.48	(0.31)	3.02	(1.35)	1.76	(1.04)
N	%	0.28	(0.04)	0.22	(0.02)	0.40	(0.12)	0.27	(0.06)	0.26	(0.11)	0.22	(0.06)
Ca	mg kg^{-1}	3354	(839)	2369	(756)	2492	(569)	3337	(2048)	1348	(1161)	503	(287)
K	mg kg^{-1}	3531	(954)	3977	(1266)	3582	(1361)	5100	(2383)	1340	(903)	1501	(460)
Mg	mg kg^{-1}	3608	(785)	3542	(698)	3340	(751)	4394	(1940)	1176	(592)	1223	(337)
P	mg kg^{-1}	294	(65)	279	(37)	240	(50)	256	(43)	219	(66)	229	(44)
S	mg kg^{-1}	251	(56)	216	(13)	199	(55)	226	(45)	255	(40)	238	(43)
Sand	%	45.0	(5.1)	46.9	(10.4)	41.5	(7.3)	42.9	(16.9)	63.0	(6.6)	62.2	(5.9)
Loam	%	16.6	(3.2)	18.7	(5.4)	20.3	(2.8)	18.0	(4.3)	16.5	(3.6)	13.8	(3.0)
Clay	%	38.3	(4.8)	34.4	(5.9)	38.2	(5.6)	39.1	(13.2)	20.5	(4.9)	23.9	(4.9)
Soil moisture	%	26.6	(2.4)	25.5	(6.0)	24.8	(3.5)	22.8	(2.7)	16.3	(3.8)	15.3	(2.0)
Organic matter	%	16.6	(1.7)	14.8	(3.0)	15.3	(2.8)	11.6	(2.2)	11.7	(4.4)	10.5	(1.3)
Cmic	mg kg^{-1}	1519	(382)	1035	(384)	1316	(237)	1134	(247)	945	(203)	929	(144)
Nmic	mg kg^{-1}	266	(54)	161	(87)	232	(64)	160	(45)	120	(30)	116	(25)
Pmic	mg kg^{-1}	51.0	(7.1)	50.4	(16.1)	44.7	(14.4)	21.3	(8.8)	17.4	(11.6)	14.7	(6.3)

(Sparks, 1996). In addition, in May 2007 we sampled 8 cm deep soil cores at each microsite to determine gravimetrical water content and estimate microbial C and N using a chloroform fumigation-extraction procedure (Brookes et al., 1985; Vance et al., 1987; Aponte et al., 2010a). More details on the characterization of microsites can be seen in the parallel studies of element cycling (Aponte et al., 2010a) and soil microbial biomass (Aponte et al., 2010c).

Forest floor removal

In addition to litterbag decomposition rates we evaluated the forest floor removal rate using the equations established by Olson (1963). The annual leaf fall (L) was collected by four traps (50 cm diameter) located under each tree. The contents were removed, and the leaf fraction was separated and dried. Two 30x30cm quadrates were used to estimate the forest floor biomass (F) by the harvesting and drying method at each sampling point. We calculated forest floor removal rates (k_1) under steady state assumption for continuous leaf fall $k_1 = L/F$. We also calculated decay rates (k_2) using Olson´s equation for discrete autumn litter fall $k_2 = -\ln(1 - L/(L+F))$.

Data analysis

We fitted biomass loss over time with two alternative decay models proposed by Wieder and Lang (1982): a single exponential decomposition model, $X = e^{-kt}$ and an asymptotic model, $X = C + (1 - C)e^{-kc \cdot t}$, where X was the proportion of remaining biomass at time t. In the single exponential model k is the decay rate. In the asymptotic model C is the fraction of the initial mass with a decomposition rate zero (i.e. the asymptote) while the remaining fraction (1–C) decomposes with a decay rate k_c. The asymptotic model implies that there is a limit value for mass loss that reflects a very stable fraction of litter that, in the time range of the experiment, decomposes extremely slowl (Berg et al., 2003). All models were fitted in R freeware (http:// www.r-project.org/) and they all constrained the proportion of initial mass remaining at time zero to be 1. Model fit was evaluated using Akaike´s Information Criterion (AIC). Models whose AIC values differed less than 2 were considered to have equivalent ability to describe the data (Burnham and Anderson, 2002).

The dynamics of elements during decay was analyzed using a polynomial regression model ($Y = B_o + B_l t + B_2 t^2$) that allows for testing both the linear and the curvilinear relationships between the chemical elements (Laskowski et al., 1995). Significance of B_1 or B_2 would be interpreted as a linear or non linear (unimodal or U-shaped) relationships. In order to compare the chemical element dynamics in litters of different decomposition rates we used Standardize Time Units (STU=k years) by multiplying it by decomposition constant k for every litter type (Laskowski et al., 1995). Thus the regression models resulted: $Y = B_o + B_l kt + B_2(kt)^2$, where Y was the concentration of the element. This allowed us to relate the concentrations of chemical elements to the stage of decomposition rather than to absolute time.

We used a t test to evaluate differences between forest sites in decomposition variables (RB, chemical element concentration) and parameters (k_c, C, B_o, $B_{l,,}$ B_2). Forest site had a significant effect thus we used analysis of covariance (ANCOVA) to investigate the effects of microsite and litter type on decomposition parameters and included forest site as a covariate. Because of the unbalanced design we first run the analysis including only the common microsite types (understorey of *Q. canariensis* and *Q. suber*) of the two forest sites, and then analysed the differences between the microhabitats within each site. Post-hoc comparisons were done using Fisher LSD test and type I error inflation resulted from repeated tests was controlled using a false discovery rate procedure (FDR) as recommended by García (2003).

To test for non-additive effects of species litter mixture on decomposition we compared the decomposition parameters for the individual and mixed species litters using ANOVA. Additionally we evaluated whether the categorical factor individual vs mixed species (mixed) explained a significant fraction of the variability of the parameters dataset assuming that decay parameters from mixed species litterbag could be predicted from the individual species. To evaluate the home field advantage theory, affinity effects were tested with individual litter species and location (*in situ* and *ex situ*). *In situ* location corresponded to individual litter species decomposing beneath their own species. *Ex situ* decomposition corresponded to individual litter species decomposing in any other microsite.

We used a model simplification approach to test for the best explanatory variable for decomposition parameters (Crawley, 2007; Dickie et al., 2009). We tested remaining biomass and decomposition rates against all measured soil properties and litter chemical composition (at time 6 months) and built a maximal model. We progressively simplified the maximal model by removing the least significant term and selecting models to minimize Akaike´s Information Criterion (AIC) values until obtain the minimal adequate model. Final significance values of terms were obtained by $\chi 2$ comparison of models excluding that term.

RESULTS

General trends of mass loss

There was a general trend of exponential loss of leaf litter mass, but with small differences between the oak species, the type of microsite where litter was incubated, and the general conditions of the forest experimental site (Fig. 1). The asymptotic exponential model was generally the best fitted of the two decay models implemented either when the models were fitted on each replicate separately (71% of 144 models fitted) or for the pooled microsite replicates (six replicates combined; 96% of 24 models fitted), based on Akaike´s Information Criterion (AIC). In no cases the best fit corresponded to the single exponential model exclusively. The exponential decay rate (k) was significantly correlated with the asymptote (C) ($r = -0.6$; $p< 0.001$) but not with the asymptotic decay rate (k_c) ($r = 0.02$; $p< 0.87$).

Litter type effects on mass loss

Leaf litter species determined significant differences in the remaining mass during the first year ($p< 0.003$) when *Q. suber* litter had higher RB values (slower decay rate) (Fig. 1, Supplementary Fig. S1). However, both oak species converged to similar values during the second year. The asymptotic decay parameters were higher for *Q. canariensis* substrate ($p< 0.002$) reflecting that litter decomposition of this deciduous species was faster during the first stage, although its fraction of slow decomposable biomass was higher (Fig. 2).

Individual and mixed species litter showed no significant differences in their remaining biomass and decay parameters. The only two exceptions were found for the remaining biomass of *Q. canariensis* at time 18 months, which was higher for the individual species (47.1%) than for the mixed species litter

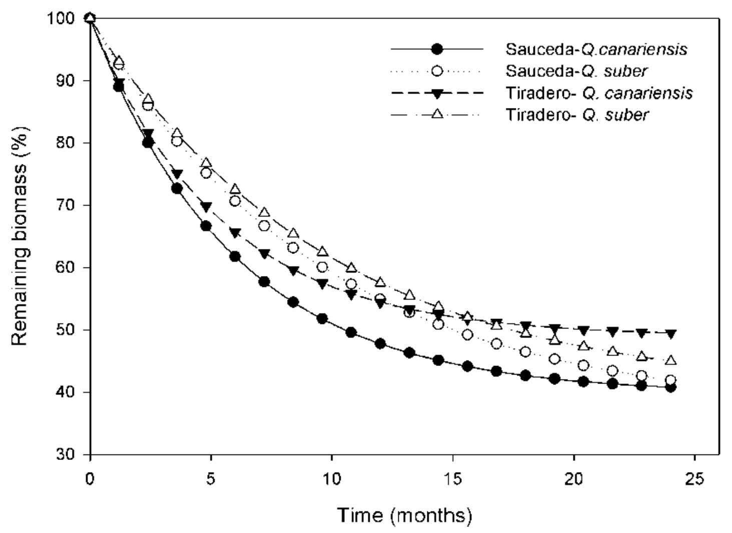

Figure 1. Predicted variation in remaining biomass (%) of leaf litter with time as a function of forest site and oak species using the fitted asymptotic model.

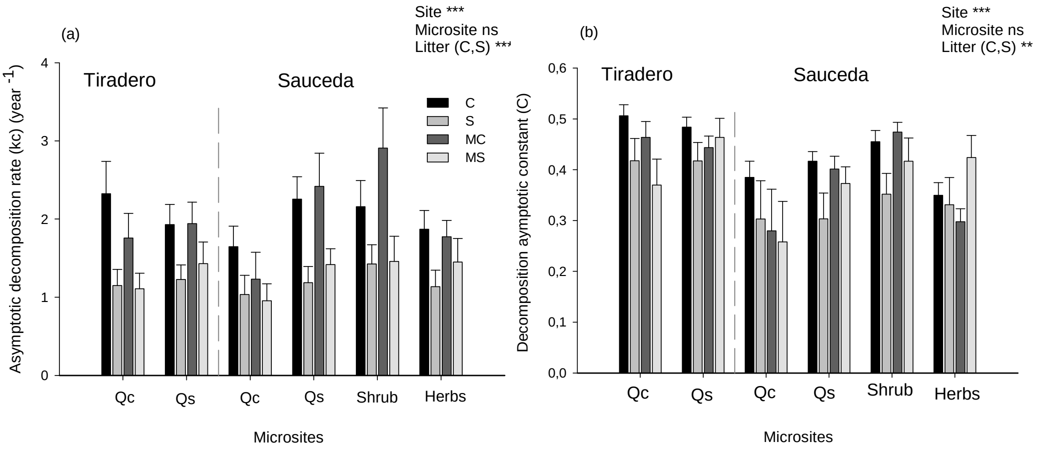

Figure 2. Decomposition constants of the single (C-*Q. canariensis*, S- *Q. suber*) and mixed (MC, MS) litters.

(42.6%, $p< 0.047$), and the asymptote value, which was also higher for the individual ($C=0.427$) than the mixed species ($C=0.384$, $p< 0.003$). The values of the remaining biomass predicted from the individual models were highly correlated ($r = 0.92$) to those recorded for the mixed species litter (Fig. 3). Species deviation, calculated as the average residual value (RB predicted-RB observed) was positive (3.6) for *Q. canari*ensis and negative (-1.96) for

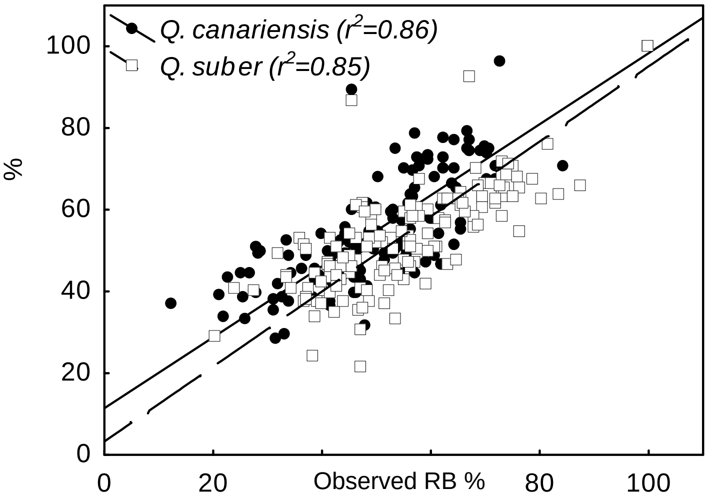

Figure 3. Observed remaining biomass (RB) of the mixed species litter in relation to the predicted RB values calculated from the individual species litter.

Q. suber. Despite there is not a strong evidence of a non-additive response to litter diversity, taken together, these results suggest that *Q. canariensis* tended to decompose faster and *Q. suber* slower in mixed species litter bags compared to individual species conditions.

Microsite type effects on mass loss

The microsite environment where litter was incubated had significant effects on remaining mass, in particular when comparing values after the second year in the Sauceda forest site (Supplementary Fig. S2). Leaf litter decomposition was slower (rendering higher RB) beneath *Q. suber* trees (41.5 %) and the dense shrubby cover (46.1 %), in comparison with beneath *Q. canariensis* trees (37.4%) and at the opens (38.2%). The highest asymptote value (C = 0.42; F= 3.533; p< 0.028) was found for litter under shrubs.

The combined analyses of interactions between litter type (oak species) and environment where litter was incubated (microsite type) showed no affinity effects on remaining biomass or decay rate. The only exception was that in the Sauceda site the decay rate of *Q. canariensis* litter was significantly slower under trees of same species (k_c = 1.20 yr^{-1}) than in other incubating environments (1.82 yr^{-1} under *Q. suber* trees and 1.99 yr^{-1} under shrubs) (F= 5.596; p< 0.005).

Table 2. Forest floor removal rates k_1 (assuming continuous litter fall) and k_2 (assuming discrete autumn litterfall) as defined by Olson (1963). Mean ± SE.

Site	Oak species	k_1			k_2		
Tiradero	*Q. canariensis*	0.20	±	0.02	0.18	±	0.01
	Q. suber	0.24	±	0.06	0.21	±	0.04
La Sauceda	*Q. canariensis*	0.33	±	0.04	0.28	±	0.03
	Q. suber	0.29	±	0.05	0.25	±	0.04

Differences between forest sites in decay rates and floor mass removal

The average proportion of remaining biomass after the 2-year decomposition period differed significantly between the two forest sites (F: 112.829; $p < 0.000$), with 30% (range 16-41%) of biomass remaining in Sauceda and 46% (range 44-50%) in Tiradero (Supplementary Fig. 2). We obtained similar results analyzing the parameters of the decomposition models. Distinct asymptote was observed for the two sites (Tiradero: $C = 0.44$; Sauceda: $C = 0.36$) but no differences were found for the decay rate.

The estimated forest floor removal rates k_1 (assuming continuous leaf fall) and k_2 (assuming discrete autumn leaf fall) were lower in Tiradero ($k_1 = 0.22$; $k_2 = 0.19$) than in Sauceda ($k_1 = 0.31$; $k_2 = 0.26$) in accordance to litterbag decay rates (Table 2). Forest floor removal rates were not related with litterbag decomposition parameters.

Leaf litter decay and nutrient dynamics

The initial concentration of Ca, Mg, P and S was higher in *Q. canariensis* than in *Q. suber* leaf litter, while that of Mn was higher for *Q. suber* (Table 3). In particular Ca and Mg had about 1.5-fold higher values in the litter of *Q. canariensis*. The polynomial model fitted on the concentration of chemical elements along the decomposition processes showed differences between elements dynamics (Table 4, Fig. 4). Calcium concentration showed a unimodal time course, with an initial period of increasing concentration followed by a second period of element loss, with curves that were approximately parallel for both oak species. Concentration of Mg remained relatively constant with time for both species. Litter P content decreased linearly for *Q. canariensis* but it remained constant for *Q. suber* (Fig. 5). Potassium showed a strong decrease in its concentration at the beginning of the decomposing period but afterwards concentration tended to be relatively constant (Table 4, Fig. 4) and net abundance remained constant (Fig. 5). Zinc and Mn showed a monotonic increase in its concentration. The two litter types significantly differed in B_0 for all chemical elements, whereas differences in the parameters B_1 ad B_2 occurred for Ca, P, Mn and Zn (Table 4). The differences between litter types observed

Table 3. Initial concentration (mean ± st. dev) of chemical elements in decomposing leaf litter. Differences between oak species were tested with one way ANOVA.

Element		*Q. canariensis*	*Q. suber*	F	P value
Ca	g kg $^{-1}$	14.84 ± 0.76	9.25 ± 0.51	221.06	0.000
K	g kg $^{-1}$	5.44 ± 0.69	4.47 ± 0.74	4.79	0.056
Mg	g kg $^{-1}$	2.11 ± 0.07	1.43 ± 0.08	172.95	0.000
P	g kg $^{-1}$	1.00 ± 0.11	0.62 ± 0.12	22.29	0.001
S	g kg $^{-1}$	1.01 ± 0.04	0.78 ± 0.05	55.15	0.000
Mn	mg kg-^{1}	864 ± 136	1075 ± 138	6.42	0.032
Zn	mg kg -^{1}	22.28 ± 6.51	17.05 ± 6.41	2.67	0.137
Cu	mg kg -^{1}	5.46 ± 0.63	4.72 ± 0.52	4.74	0.057
N*	%	0.95 ± 0.18	0.91 ± 0.11	0.28	0.759

* data from Aponte et al. (2010a)

Table 4. Relation between element concentrations (Y) and standardized time (ST) for leaf litter of *Q. canariensis* (C) and *Q. suber* (S); regression model: $Y=B_o+B_1*ST+B_2*ST^2$; the significance of parameters is indicated (*** p<0.001, ** p<0.01, *p<0.5, ns not significant).

Element	Litter	B_o		B_1		B_2		R^2
Ca	C	$16.66^{a} \pm 0.93$	***	$27.12^{a} \pm 3.52$	***	$-14.99^{a} \pm 2.84$	***	0.38 ***
	S	$9.33^{b} \pm 0.56$	***	$19.50^{b} \pm 2.39$	***	$-9.09^{b} \pm 2.11$	***	0.53 ***
K	C	$4.747^{a} \pm 0.141$	***	$-9.537^{a} \pm 0.536$	***	$5.736^{a} \pm 0.433$	***	0.73 ***
	S	$3.990^{b} \pm 0.122$	***	$-8.658^{a} \pm 0.518$	***	$5.962^{a} \pm 0.457$	***	0.68 ***
Mg	C	$2.106^{a} \pm 0.077$	***	$-0.258^{a} \pm 0.293$	ns	$-0.105^{a} \pm 0.236$	ns	0.10 ns
	S	$1.391^{b} \pm 0.055$	***	$-0.194^{a} \pm 0.233$	ns	$0.396^{a} \pm 0.206$	ns	0.08 ns
P	C	$0.986^{a} \pm 0.031$	***	$-0.425^{a} \pm 0.117$	***	$0.166^{a} \pm 0.095$	ns	0.20 ns
	S	$0.596^{b} \pm 0.027$	***	$0.184^{a} \pm 0.113$	ns	$-0.044^{a} \pm 0.100$	ns	0.08 ns
S	C	$1.058^{a} \pm 0.057$	***	$1.288^{a} \pm 0.215$	***	$-1.059^{a} \pm 0.173$	***	0.18 ns
	S	$0.751^{b} \pm 0.046$	***	$1.428^{a} \pm 0.196$	***	$-0.998^{a} \pm 0.173$	***	0.28 ns
MN	C	$0.798^{a} \pm 0.071$	***	$0.895^{a} \pm 0.269$	**	$-0.318^{a} \pm 0.217$	ns	0.20 ns
	S	$1.038^{b} \pm 0.061$	***	$0.049^{a} \pm 0.258$	ns	$0.487^{a} \pm 0.228$	*	0.25 ns
ZN	C	$0.028^{a} \pm 0.008$	***	$0.122^{a} \pm 0.031$	***	$-0.012^{a} \pm 0.025$	ns	0.43 ns
	S	$0.015^{b} \pm 0.006$	*	$0.117^{b} \pm 0.025$	***	$-0.033^{b} \pm 0.022$	ns	0.40 ns
CU	C	$0.006^{a} \pm 0.000$	***	$0.014^{a} \pm 0.002$	***	$-0.007^{a} \pm 0.001$	***	0.41 ***
	S	$0.005^{b} \pm 0.000$	***	$0.015^{a} \pm 0.002$	***	$-0.01^{a} \pm 0.00$	***	0.58 ***

^{a} Significant differences p<0.05 between litter types

for element net loss indicated a higher and faster nutrient release (for Ca, Mg, P and S) from *Q. canariensis* litter (Fig. 5). The microsite type had no effect on any regression parameters but it affected chemical element concentration and net element abundance, with values generally higher beneath *Q. canariensis* and shrub than beneath *Q. suber* and herbs (See Supplementary Fig. S3). We found no interaction of species in mixed litterbags: i.e. no significant differences were found between B_0, B_1 and B_2 of the individual and mixed species litter for any chemical element.

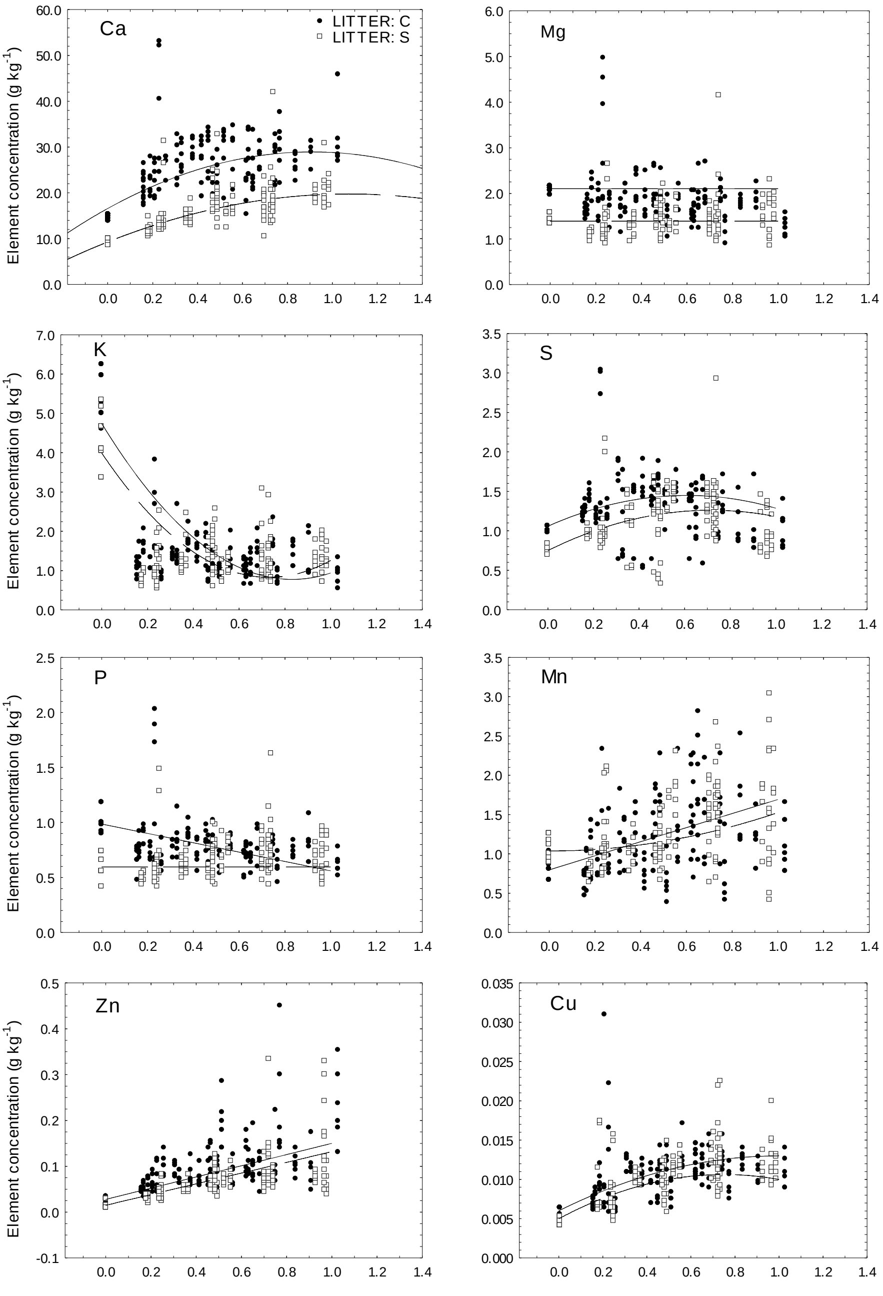

Figure 4. Dynamics of the concentration of chemical elements in the decomposing leaf litter of *Q. canariensis* (C; solid line and filled circles) and *Q. suber* (S; dashed line and hollow squares). Time is expressed in standardized time units (STU = time (yr) x decomposition constant k).

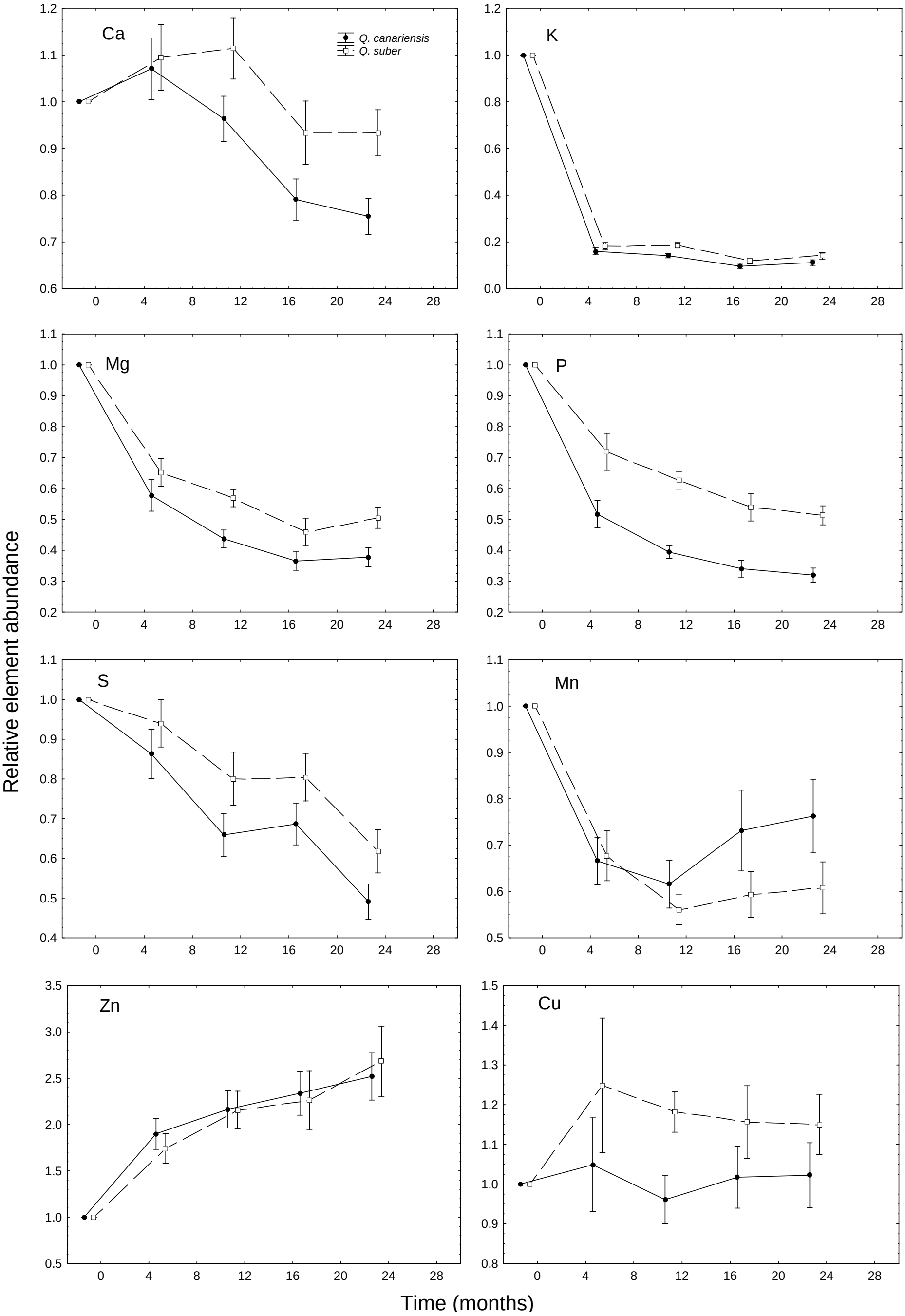

Figure 5. Dynamic of the net element immobilization into decomposing litter of *Q. canariensis* (solid lines and filled circles) and *Q. suber* (dashed lines and hollow circles) along the 2-year experiment. Values are relative to initial element abundance.

Predictors of litter decomposition

In the regression analysis, Ca concentration in litter and soil emerged as the best predictor for decay parameters (Supplementary Table S1). Soil Ca was negatively related to the asymptote value ($p< 0.023$) and it explained 10.2% of the variance of C when it was estimated for each replicate and 45% when it was estimated from the replicates pooled by microsite. Regression fit increased to $r^2= 0.61$ when only the microsites beneath the oak trees were included. Soil Ca was also the best predictor for the remaining biomass after two years ($p< 0.001$, $r^2=0.21$). The decay constant was best predicted by litter Ca concentration, measured at 6 months ($p< 0.001$). Both soil and litter content were equally the best predictors for forest floor removal rates based on AIC ($p< 0.006$; $r^2 = 0.20$).

DISCUSSION

We parameterized the leaf litter decomposition using two decay models, a negative exponential model that implies a continued mass loss, and an asymptotic model that regards substrate as consisting of a component decomposing exponentially and a hardly decomposable component (Berg et al., 2003). In most cases, the asymptotic model had a better fit than the single exponential model. The asymptotic model is less commonly used to model litter mass loss than single negative exponential model despite some researchers have argued against the latter (e.g.Wieder and Lang, 1982; Ostrofsky, 2007). The biological realism of the asymptotic model could be questioned since no fraction of plant litter is completely indecomposable. However this model accurately describes the data obtained under certain experimental manipulations (e.g. when soil fauna is excluded) and during the period over which a particular study is conducted (De Angelis et al., 2000; Kurz et al., 2000).

Leaf litter type and microsite environment affect mass loss

During the initial phase of decomposition leaf litter of *Q. canariensis* showed a faster decay (higher k_c, lower RB) than leaf litter of *Q. suber*. In this early period we observed also a microsite effect on *Q. canariensis* litter, which decomposed slower beneath trees of its own species than beneath other types of microsites. During the second phase of decay the fraction of hardly decomposable litter of *Q. canariensis* was higher than the fraction of *Q. suber* and the decomposition of both types of litter proceeded further beneath *Q. canariensis* cover. In both forest sites decomposition parameters were best predicted by Ca content in litter (k_c), in soil (C) or in both (k_1,k_2) suggesting that differences in Ca concentration in litter and soil explain a significant fraction of the variation in the decomposition process in the studied forests. We can speculate than in these forests oak species exert some control on the different phases of decomposition *via* litter calcium content.

A main difficulty to identify the key variables that control litter decomposition is the high correlation existing between the chemical and structural traits of leaf litter (Garten, 1976; Wright et al., 2004; Aponte et al., 2010a). Commonly litter nitrogen and lignin content as well as tensile strength and toughness have been identified as the best predictors for mass loss rates (Pérez-Harguindeguy et al., 2000; Sariyildiz and Anderson, 2003; Wieder et al., 2009). Results from previous studies on the studied oak species have shown almost no differences in the leaf fall N content between them (0.95 % in *Q. canariensis*, 0.91 % in *Q. suber*; Aponte et al., 2010a); a similar lignin content (10.5% for *Q. canariensis* and 9.0% for *Q. suber*) and a slightly higher soluble polyphenols for Q. *canariensis* (13.3%) than for *Q. suber* (10.6%) (Gallardo and Merino, 1993) that would not support the observed patterns. Nevertheless more information about their carbon quality (e.g. non-structural carbohydrates, phenolics and tannins) would be needed to dissect the mechanisms driving litter decomposition in these oaks species (Hättenschwiler and Jørgensen, 2010).

Plant species might modulate environmental factors such as soil temperature and moisture and influence on soil fertility, soil microbial biomass and soil fauna through the quality of their nutrient return, generating distinct incubation conditions that indirectly affect decomposition dynamics (Chadwick et al., 1998; Prescott, 2002; Hättenschwiler and Gasser, 2005; Hobbie et al., 2006). We recorded a low remaining biomass in the open microsites, despite their low fertility values, similar to that found beneath *Q. canariensis*. It is possible that decomposition in these microsites might be driven by photodegradation, which may render a significant mineralization when solar radiation is not intercepted (Austin and Vivanco, 2006).

During the last decade, there has been an increasing number of studies that have found a strong relationship between higher litter and soil calcium content (and the related pH) and greater decomposition rates in temperate forest (Chadwick et al., 1998; Berg, 2000; Reich et al., 2005; Hobbie et al., 2006) and among a wide range of subartic flora (Cornelissen et al., 2006). The specific conditions of nutrient limitation in each ecosystem might determine the relevance of chemical elements in the decomposition process (Hobbie and Vitousek, 2000; Kaspari et al., 2008). Acidic soils as those found in our study sites have a limited Ca availability that could be related to its significant role in the decomposition process. Calcium has been previously reported as a key element for soil organisms. Soil conditions induced by certain tree species enriching in Ca have been related to a higher microbial activity, since the stimulation of microflora lignin-degrading and pectin-degrading enzymes requires Ca as a cofactor (Eriksson et al., 1990; Berg et al., 2003). In another example, tree species rich in calcium were associated with increased soil Ca and pH, increased earthworm abundance and diversity, and higher decomposition rates (Reich et al., 2005; Hobbie et al., 2006). In a parallel study, conducted in the same forest sites, we showed a shift in the ectomycorrhizal fungal community induced by distinct oak litter Ca (Aponte et al., 2010b) that might be related to distinct decomposing capacities (Conn and Dighton, 2000; Buée et al., 2007).

Decomposition process at the two forest sites seemed similar in the early stages but proceeded further at Sauceda, where remaining biomass was lower (30%) than in Tiradero (46%). The significantly higher soil Ca amounts recorded in Sauceda than in Tiradero could be a potential reason for this difference in decomposition limit value. Several studies related site fertility and decomposition rates (Prescott, 1996; Hobbie and Vitousek, 2000; Santa Regina, 2000) particularly in the later stages of decomposition (Berg et al., 2003). Nevertheless, the faster and extended decay in Sauceda as compared to Tiradero might also be attributed to regional differences such as the higher temperature and rainfall, or the higher UV radiation in the less dense forest (Austin and Vivanco, 2006; Hobbie et al., 2006; Wieder et al., 2009).

Differences in decay rates are determined by leaf litter quality (varying between deciduous and evergreen oaks) and by the environmental conditions in the incubating microsite; we suggest that these conditions are partly influenced by the tree in a feed-back process (Aponte et al., 2010a). However, we observed no positive interaction between substrate and microsite that could suggest species affinity effects as those expected by the home advantage theory (Vivanco and Austin, 2008; Ayres et al., 2009). In contrast, deciduous leaf litter decomposed faster in environments other than its own, maybe because it would represent a Ca input in Ca-poor microsites.

We found a non-additive response of species litter mixture opposite to the synergistic effect we had hypothesized. We observed that decomposition of *Q. canariensis* was enhanced, and that of *Q. suber* tended to be retarded in mixed litterbags compared to individual species litterbags. Gartner and Cardon (2004) reviewed decomposition in mixed species leaf litter. They found that 67% of all mixture tested (108 out of 162 in 30 reviewed papers) exhibited non-additive response and synergistic effects were more common than antagonistic ones. The negative interaction observed between the two oaks litter might reflect the decomposers preference for *Q. canariensis* leaves over *Q. suber* as a result of their distinct litter quality (Cornelissen et al., 1999). Nevertheless, further study is needed to clarify this response.

Leaf litter decay and nutrient dynamics

Litter decomposition dynamics differed among chemical elements, from those that were rapidly released (K), through those with stable concentrations (P, Mg) up to those showing a constant increase (Zn). All chemical elements exhibited similar relative mobility in the two litter types except for Mn and Cu.

On average elements were released in the following order: K>Mg>P>Mn>S>Ca>Cu>Zn as can be deduced from Fig. 5. The fast decrease of concentration of K in decomposing litter is usually reported in a broad range of forest ecosystems (Attiwill, 1968; Berg, 1986; Blair, 1988). In a similar study Laskowski et al. (1995) also found a significant increase in the absolute amount of Zn, together with Fe, Pb and Cd, which was related to throughfall input.

Different patterns of litter nutrient release were observed between our forest sites and other temperate forests. For example, in other studies P amount increased at the initial stages of decomposition, followed by a release (Staaf and Berg, 1982; Maheswaran and Attiwill, 1987); however, we observed a net decrease and constant or decreasing concentrations of P in litter of both oaks. These studies also showed an absolute decrease of Ca, Mg and Mn, while their concentrations either remained constant (Ca) or decreased (Mg, Mn), opposite to the patterns reported here. Distinct patterns in the dynamics of particular chemical elements in various forest ecosystems reflect different nutrient availability to decomposers, with limiting nutrients usually accumulating during the initial stages of decomposition (Swift et al., 1979; Staaf and Berg, 1982). The observed immobilization of Ca in both litter types supports our hypothesis that Ca is a main limiting element for decomposition process in these forests on acidic, Ca-poor soils. Similar patterns of Ca dynamics in decomposing pine, beech and Oak-hornbeam litters have been also reported (Laskowski et al. (1995); authors suggested that the mechanisms behind the initial immobilization of Ca reflected a nutrient demand of decomposers organism. Concentration of Ca in our two litter types started to decline at the same stage of decomposition (Standard time) suggesting that at that stage of decomposition the potential mechanism would be directly related to organic matter decomposition rate. Calcium, which is confined to structural components of plant tissues, is relatively immobile during initial stages of decomposition and could be released only as the decomposition of structural compounds proceeds at latter stages of decomposition associated to cell decomposing fungi (McClaugherty and Berg, 1987; Blair, 1988; Laskowski et al., 1995).

In the study forests, oak species have both direct –via leaf fall quality - and indirect –via soil conditions- effects on decomposition, with *Q. canariensis* producing high quality litter that decomposes faster and generating an environment that allowed decomposition to proceed further. In addition the analysis of the chemical decomposition patterns showed that for most macronutrients (Ca, Mg, P and S) relative net nutrient release was higher for *Q. canariensis*, which also showed the higher initial nutrient concentrations. These results are consistent with the functional strategies characteristics of evergreen and deciduous tree species. Deciduous trees are nutrient demanding species which fast growth depends on nutrient availability, thus it is likely that they are associated with high decomposition rates and fast nutrient cycles. Evergreen species in contrast produce low quality leaf litter that decomposes slowly and increases carbon sequestration, keeping nutrient mineralization low and generating nutrient-poor environments where they have competitive advantage (Hobbie, 1992; Aerts, 1995; Cornelissen et al., 1999; Aponte et al., 2010a). The species composition of these forests has been long influenced by climate conditions and forest management (Urbieta et al., 2008). In the light of our results, the predicted shift in species composition derived from the ongoing global change may have significant consequences in leaf litter decomposition dynamics, nutrient cycling and, ultimately, on ecosystem functioning (Schröter et al., 2005; Bates et al., 2008).

CONCLUSIONS

We have investigated the mass loss and the chemical dynamics of decaying leaf litter of two co-occurring oak species with contrasting leaf habit in a Mediterranean forest and evaluated the relative importance of the direct and indirect effects of trees on the decomposition process. Litter mass loss was best described by an asymptotic decay model that differentiated a first decomposition period with constant decay rate (k_c) and a second period where litter mass loss tended to zero. Decay rate was affected by litter type, apparently mediated by Ca content, while microsite conditions influenced the decomposition limit values possibly through distinct Ca availability. *Q. canariensis* produced enriched litter that decomposed faster and its environment promoted further litter decomposition and lower limit values. Additionally forest floor removal rate, which was estimated independently from the litterbag experiment, also differed between oak species and it was best predicted by soil and litter Ca contents. We observed a non-additive response of species litter mixture, resulted from an antagonistic effect possibly related to the higher quality of *Q. canariensis* litter. In contrast to what we had hypothesized, we found no affinity effect between litter and microsite, thus non supporting the home field advantage theory. The rates of nutrient release were very variable among chemical elements, from rapid loss (K) to constant increase (Zn). Calcium was immobilized during the first stage of decomposition, probably due to a high decomposers demand, while released during latter stages of decomposition in both litter types.

Altogether these results suggest that in these forests oak species affect decomposition both through their distinct litter quality and through their soil environment resulting from long term tree-soil interactions.

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SUPPLEMENTARY MATERIAL

Figure S1. Remaining biomass (%) observed for the single (C-*Q. canariensis*, S- *Q. suber*) and mixed (MC, MS) species litter in the two study sites. Differences between litter types are shown (* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$).

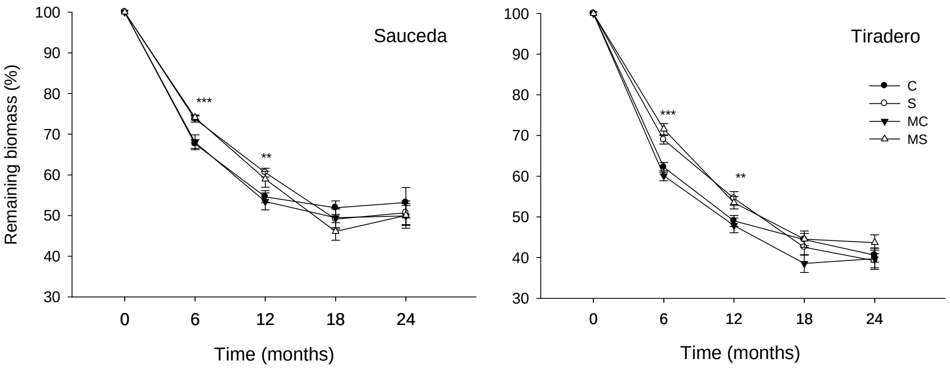

Figure S2. Remaining biomass (%) after two years of *Q. canariensis* and *Q. suber* leaf litter in the four microsites at Sauceda site. One standard error of the mean is presented (* $p < 0.05$, ns not significant).

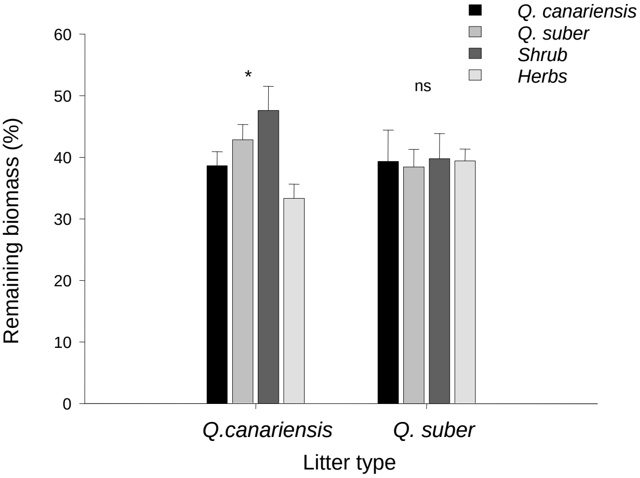

Figure S3. Average concentration of chemical elements after the 2-year experiment for each site and microsite (*** $p < 0.001$; ** $p < 0.01$; $p < 0.05$; ns not significant differences).

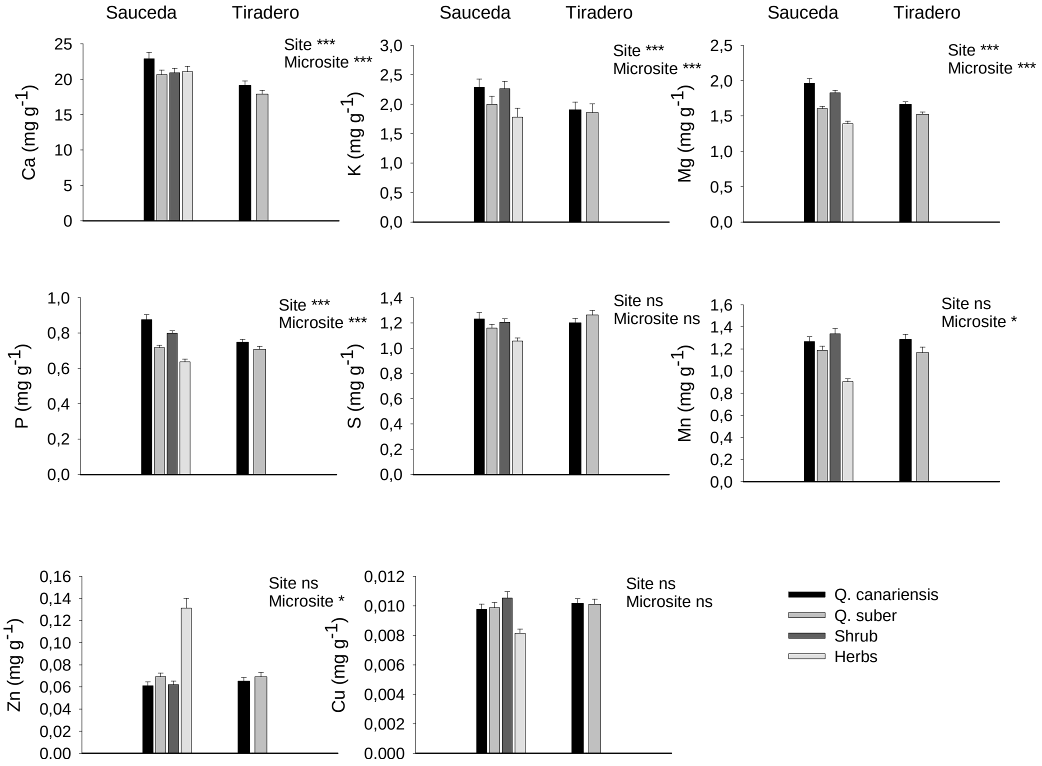

Table S1. Results of the model selection for decay parameters (k_c, C), remaining biomass after 24 months (RB_{24}), and forest floor removal rates (k_1, k_2). Minimal adequate model selection was based on lowest AIC value which resulted in retaining the predictors term with significant p (χ^2). The p (χ^2) values are a χ^2 comparison of models excluding the predictor term. The sign of the relationship between selected variables and decay parameters (- or +) is indicated.

Response variable	Minimal adecuate model vs null model	AIC	p (χ^2)	R^2	F	p (F)
k_c [a]	Litter Ca (+)	366.55	**0.0005**	0.08	13.69	0.0001
	Null	377.05				
C [a]	Soil Ca (-)+ Soil P (-)	-201.52	**0.0001**	0.13	10.98	0.0001
	Null	-189.55				
k_c [b]	Litter Ca (+)	185.29	**0.0001**	0.19	32.27	0.0001
	Null	212.46				
C [b]	Soil Ca (-)	-404.46	**0.0231**	0.45	18.03	0.0001
	null	-401.31				
RB_{24}	Soil Ca (-)	1040.9	**0.0001**	0.21	37.02	0.0012
	null	1072.1				
k_1	Soil Ca (-)	-69.62	**0.0012**	0.23	11.33	0.0018
	Litter Ca (+)	-68.51	**0.0020**	0.21	10.05	0.0031
	Soil Ca (-)+Litter Ca (+)	-68.44	**0.0035**	0.25	6.039	0.0053
	null	-61.12				
k_2	Soil Ca (-)	-90.93	**0.0026**	0.20	9.63	0.0036
	Litter Ca (+)	-90.14	**0.0041**	0.19	8.71	0.0085
	Soil Ca (-)+Litter Ca (+)	-89.71	**0.0074**	0.22	5.15	0.0106
	null	-83.89				

[a] model fit to decay parameters estimated for each replicate

[b] model fit to decay parameters for pooled microsite replicates

Capítulo 6.

Factores que afectan a la masa microbiana del suelo en un bosque de quercíneas mediterráneo

Este capítulo reproduce el siguiente manuscrito:

Aponte, C., Marañón, T., García, L.V. 2010. *Microbial C, N and P in soils of Mediterranean oak forests: influence of season, canopy cover and soil depth.* Biogeochemistry (en prensa) DOI 10.1007/s10533-010-9418-5.

Capítulo 6. **Factores que afectan a la masa microbiana del suelo en un bosque de quercíneas mediterráneo**

RESUMEN

El patrón climático estacional característico de los ecosistemas mediterráneos puede condicionar el efecto que los factores ambientales tienen sobre la masa microbiana del suelo y los ciclos de liberación e inmovilización de nutrientes. En este trabajo hemos estudiado el efecto que la estación, la cubierta vegetal y la profundidad del suelo tienen sobre el carbono, nitrógeno y fósforo microbiano del suelo en dos rodales de bosque mediterráneo empleando un procedimiento de fumigación-extracción.

Los valores medios estimados para la masa microbiana fueron 820 µg C g $^{-1}$, 115 µg N g $^{-1}$ y 19 µg P g $^{-1}$, que supusieron un 2.7%, 4.7% y 8.8% de las cantidades totales de C, N y P en el suelo superficial. El N y P microbiano fueron 10 veces mayores que las fracciones de N y P inorgánico disponible para las plantas. Los valores de C microbiano difirieron entre los dos rodales estudiados, pero en cada rodal los valores no variaron entre estaciones. Ambos el N y P microbiano e inorgánico mostraron valores máximos en primavera y mínimos en verano, relacionados con la humedad del suelo. Los valores microbianos difirieron significativamente entre los suelos superficiales con distinto tipos de cobertura vegetal, pero solo en condiciones ambientales favorables (primavera) y no durante el verano. Los contenidos microbianos decrecieron hasta dos veces con la profundidad del suelo. Los ratios de nutrientes microbianos (C/N, C/P y N/P) variaron con la estación y la profundidad del suelo. El régimen de humedad del suelo, íntimamente relacionado con la estacionalidad, podría ser un factor clave en el crecimiento de la masa microbiana en los bosques estudiados.

Este trabajo muestra que en sistemas forestales mediterráneos la interacción entre las estaciones, el tipo y estructura de la vegetación y las propiedades del suelo afectan a la inmovilización microbiana de nutrientes y podría influir en los ciclos biogeoquímicos de carbono, nitrógeno y fósforo.

Microbial C, N and P in soils of Mediterranean oak forests: influence of season, canopy cover and soil depth

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Abstract

In Mediterranean ecosystems the effect of aboveground and belowground environmental factors on soil microbial biomass and nutrient immobilization-release cycles may be conditioned by the distinctive seasonal pattern of the mediterranean-type climates. We studied the effects of season, canopy cover and soil depth on microbial C, N and P in soils of two Mediterranean forests using the fumigation-extraction procedure. Average microbial values recorded were 820 µg C g $^{-1}$*,115 µg N g* $^{-1}$ *and 19 µg P g* $^{-1}$*, which accounted for 2.7%, 4.7% and 8.8% of the total pools in the surface soil, respectively. Microbial N and P pools were about 10 times higher than the inorganic N and P fractions available for plants. Microbial C values differed between forest sites but in each site they were similar across seasons. Both microbial and inorganic N and P showed maximum values in spring and minimum values in summer, which were positively correlated with soil moisture. Significant differences in soil microbial properties among canopy cover types were observed in the surface soil but only under favourable environmental conditions (spring) and not during summer. Soil depth affected microbial contents which decreased twofold from surface to subsurface soil. Microbial nutrient ratios (C/N, C/P and N/P) varied with seasons and soil depth. Soil moisture regime, which was intimately related to seasonality, emerged as a potential key factor for microbial biomass growth in the studied forests.*

Our research shows that under a mediterranean-type climate the interaction among season, vegetation type and structure and soil properties affect microbial nutrient immobilization and thus could influence the biogeochemical cycles of C, N and P in Mediterranean forest ecosystems.

Key words*: Microbial biomass, nitrogen, nutrient immobilization, phosphorus, plant-soil interactions, seasonal dynamics, vegetation cover*

INTRODUCTION

Soil microbes play an essential role in the main biogeochemical transformations of organic matter and in soil fertility (Jenkinson and Ladd, 1981). During the mineralization process, an important fraction of the C, N and P in the decomposing residues is immobilized in the microbial biomass as part of their cellular constituents (e.g. phospholipids and proteins), and then released upon microorganism death (Anderson and Domsch, 1980; Jonasson et al., 1999). The capacity of microorganisms to act both as a sink and a source of nutrient resources is particularly relevant for plant nutrition since most of the annual N and P requirements of land plants are supplied from the decomposition of organic matter in the soil (Singh et al., 1989). Changes in biomass, physiology, composition and activity of soil microbes may affect their functional capacity and thus the ecosystem geochemical processes (Balser and Firestone, 2005; Crenshaw et al., 2008).

Both aboveground and belowground factors affect microbial biomass and, therefore, nutrient availability (García et al., 2002; Schade and Hobbie, 2005). Vegetation structure and composition exert a control on microbial growth through litter quality and quantity and root exudates that determine the input fluxes of labile C and nutrients (N, P) (Fisk and Fahey, 2001; Kara et al., 2008). Soil chemical and physical characteristics, like soil organic matter and soil structure and texture, may also constrain microbial developments (Hassink, 1994), and the variability of these properties along soil profile is reflected in the microbial communities (Fierer et al., 2003). Climatic conditions have a direct effect on microbial communities through soil moisture and temperature (Ley et al., 2004; Nielsen et al., 2009), but they may also have an indirect effect thought interactions with other factors such as vegetation, topography and landscape (Myers et al., 2001; Malchair and Carnol, 2009). Temporal patterns of microbial growth and nutrient immobilization-release cycles usually reflect seasonal changes (but see Raubuch and Joergensen, 2002) although such a response vary among ecosystems depending on their particular moisture and temperature regimes (Bohlen et al., 2001; Ley et al., 2004; Nielsen et al., 2009).

In this study we investigated the effects of several abiotic and biotic factors on the soil microbial C, N and P in Mediterranean oak forests. Mediterranean ecosystems are subjected to a marked seasonality that imposes a severe summer drought after a favorable rainy autumn and spring, that is reflected in soil microbial dynamics (Quilchano and Marañón, 2002). Vegetation traits that overcome mediterranean summer drought, such as long-lived and hard leaves (sclerophylly), influence litter quality and quantity and thus decomposition processes (Gallardo and Merino, 1993; Rutigliano et al., 2004). In addition Mediterranean forests have large environmental heterogeneity (species composition, canopy structure, soil properties) that might affect microbial spatial patterns (Joffre et al., 1996; Quilchano et al., 2008). The interplay of driving factors on the microbial dynamics – climate, vegetation and soil – is key to understanding the biogeochemical cycles in Mediterranean forests (Bohlen et al., 2001); however there are few studies on microbial biomass and nutrient dynamics in this type of ecosystem (Gallardo et al., 2009).

Mediterranean ecosystems, as other areas under semiarid climate conditions, are predicted to experience warmer and drier conditions due to climate change (Bates et al., 2008). Information on how microbial nutrients immobilization-release cycles are affected by environmental factors under the characteristic Mediterranean seasonal pattern could increase our understanding on how climate change may affect microbial controls over nutrient availability in this and other ecosystems and regions.

Our main objective was to investigate the main factors affecting microbial C, N and P content in Mediterranean forest soils. In particular we tested the following hypotheses: i) under mediterranean climatic conditions there are seasonal patterns with higher microbial growth and nutrient retention during the warm and wet season (spring), and a decline in microbial population and nutrient retention during the hot and dry season (summer); ii) distinct vegetation cover and composition affect soil microbial properties, with higher microbial carbon under deciduous trees which have a nutrient richer litterfall; and iii) microbial nutrient content is higher in surface than in the subsurface soil. We also tested whether these soil microbial patterns are consistent in different forest sites and analyzed the interactions between these factors.

METHODS

Site description

This study was conducted in the oak forests of Aljibe Mountains, near the Strait of Gibraltar, in southern Spain (Fig. 1). Acidic, nutrient-poor soils are developed over Oligo-Miocene sandstone bedrock that is frequently interspersed with layers of marl sediments yielding soils richer in clay. Climate is subhumid Mediterranean-type with warm, dry summers, and humid, mild winters. Temperatures average 24ºC in summer and 8.5ºC in winter. Mean annual rainfall varies from 701mm to 1331mm, depending on the mountain topography, and most (95%) of it falls from October to May. Vegetation is dominated by sclerophyllous evergreen cork oak (*Quercus suber* L.), mixed with the winter-deciduous Algerian oak (*Q. canariensis* Willd.), which is locally abundant in the valley bottoms (Urbieta et al., 2008). Both oak species differ in their leaf fall and litter quality: *Q. canariensis* has a higher nutrient content (Ca, K, Mg, S) and a lower C/N ratio compared *Q. suber*, what induces distinct soil conditions via litter decomposition (Aponte et al., 2010). There is a diverse shrubby and arborescent understorey of *Phillyrea latifolia, Erica spp*. and *Pistacia lentiscus*. See detailed description in Ojeda et al. (2000).

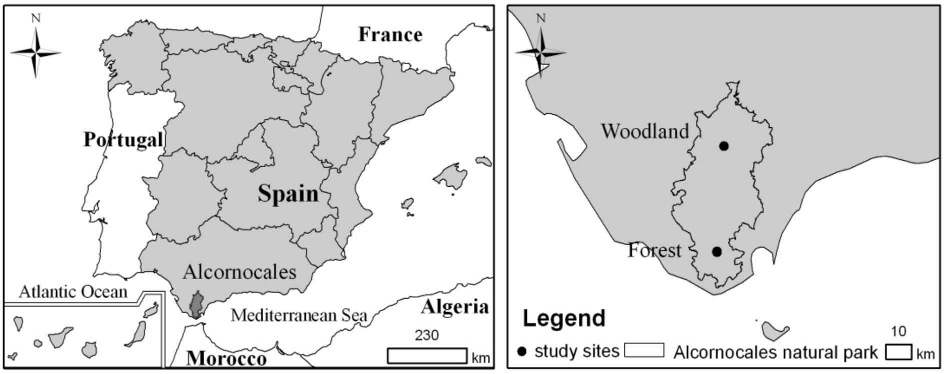

Figure 1. Location of the study area and the two forest sites in the Iberian Peninsula

Table 1. Climate and vegetation structure of the studied forest sites. Sources are the AEMET for climate and Pérez-Ramos et al. (2008) for vegetation.

	Forest	Woodland
Mean Rainfall (mm)		
Spring	216.8	258.9
Summer	21.1	28.0
Autum	262.3	319.2
Winter	472.2	526.4
Total	972.3	1132.4
Mean Temperature (ºC)		
Annual	16.6	15.5
Minimum	4.1	1.8
Maximum	23.4	23.6
Vegetation structure		
Density of trees (stems.ha^{-1})	768.8	218.8
Density of arborescent shrubs (stems.ha^{-1})	256.3	450.0
Basal area (m^2 ha^{-1})	47.0	22.1
Leaf area index (m^2 m^{-2})	2.26	1.84

For this study two forest stands of different structure, 40 km apart, were selected within the study area. The first one, at San Carlos del Tiradero (36º 9’ 46’’ N 5º 35’ 39’’ W) hereafter called "Forest", was located in the south of the study area close to the coast, at 335–360 m a.s.l. on a NE slope. The second stand hereafter called "Woodland" was located at La Sauceda (36º31’54’’N 5º34’29’’W) and stood inland, in the north of the area, at 530–560 m a.s.l. on a NW slope. The Forest site had a higher density of trees and a close canopy cover while the Woodland site had higher canopy heterogeneity and fewer trees mixed with abundant shrubs and gaps (Table 1). Both sites presented a large heterogeneity in their chemical and physical soil characteristics; see details on the forest sites in Quilchano et al. (2008) and Pérez- Ramos et al. (2008).

Field sampling

Soil samples were taken in spring (May-June), summer (September) and autumn (December) 2007, and spring (May) 2008. Soils cores were extracted at two depths (0-8 cm and 8-16 cm) using an auger. Each sample was composed of four subsamples. At the Woodland site four microhabitats corresponding to different vegetation cover types were studied i.e. soil beneath the canopy of *Q. canariensis* (Qc), beneath *Q. suber* (Qs), under shrubby cover (S) and in gaps with grass cover (G). At the Forest site, soils beneath two types of canopy cover – *Q. canariensis* and *Q. suber* – were studied. To minimize the effect of the inherent site variability ten replicates of each microhabitat were sampled at each season and soil depth, which made up a total of 480 samples. Two 30x30cm quadrates were used to assess the thickness of the litter layer, using a folding rule; and the litter biomass, by the harvesting and drying method (expressed as kg dry mass m^{-2}) at each sampling point. This sampling design allowed us to assess the effects of three factors: season, vegetation cover type and soil depth on microbial C, N and P, and to test their consistency between the two sites.

Laboratory analysis

Soil samples were brought to the laboratory in an ice-box and they were stored at 4ºC for a maximum of three days. Stones, roots and other recognizable plant parts contained in the samples were removed and the soil was homogenized through a 2mm sieve. A subsample of 1g was used to determine the water content gravimetrically by weighing the fresh and dried (105ºC) soil. The same subsample was then incinerated for 4 hours at 550ºC to determine the soil organic matter content by calcination method (Sparks, 1996).

Microbial C, N and P were estimated in the fresh soils using a chloroform fumigation-extraction procedure (Brookes et al., 1982; Brookes et al., 1985; Vance et al., 1987). Two soil subsamples (10g and 5g) were extracted using 50ml of 0.5M K_2S0_4 or 50 ml of 0.025N HCl + 0.03N NH_4F for subsequent determination of microbial C and N or microbial P, respectively. The other two soil subsamples (10g and 5g) were fumigated with chloroform for 24h in a vacuum desiccator, followed by the same extraction procedure as the unfumigated samples. The soil extracts were frozen until their C, N, P content were measured. The C and N in the fumigated and unfumigated soil extracts were determined using a Total Dissolved Organic Carbon and Nitrogen Analyzer (TOC-Vesh). Microbial C and N were estimated as the difference in K_2SO_4-extractable dissolved organic carbon or nitrogen between fumigated and unfumigated soils using as extractability correction factors: K_c= 0.45 for C and K_n= 0.40 for N (Jonasson et al., 1996; Rinnan et al., 2008). Available P in NH_4F soil extracts was measured using the Bray Kurtz method (Bray and Kurtz, 1945). Microbial P was estimated as the difference in available P between the fumigated and the unfumigated soil using a correction factor K_p= 0.40 (Brookes et al., 1982).

Soil ammonium and nitrate and total C, N and P contents were analysed in the unfumigated soils. Inorganic nitrogen (NH_4^+ and NO_3^-) was extracted using 2M KCl and determined by distillation–titration in a Bran-Luebre Autoanalyzer. Soil total C and N were estimated using an Autoanalyzer LECO. Soil total P was determined by acid digestion and ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer) analysis (Sparks, 1996). Concentrations of the elements are given on a dry weight basis (105ºC).

Data Analysis

To evaluate the effects of seasonality and canopy cover type on the soil microbial properties of the two forest sites at the two soil depths we used repeated-measures analysis of variance (ANOVA) on a split-plot design with sampling season defined as a within-factor of four levels, each one divided into two forest types (large units in the split-plot design) and each one further divided into canopy cover types (split-plots). Because of the unbalanced design, we first run the analysis including only the common cover types (*Q. canariensis* and *Q. suber*) of the two forest sites, and then analyzed the differences between the microhabitats within each site. Variables were transformed (log, arcsine) to meet necessary assumptions of normality and homocedasticity. Post-hoc comparisons were done using Fisher LSD test and type I error inflation resulted from repeated tests was controlled using the False Discovery Rate (García, 2003). This technique was preferred over Bonferroni-related procedures that notably increase power losses. General trends of soil microbial C, N and P values were related to other soil features (moisture, organic matter and inorganic nutrients) by correlation analysis.

RESULTS

Soil patterns

There were significant differences in soil properties between canopy cover type and soil depth, and also between sites (Appendix 1). Soil water content varied across season attaining minimum values in summer (average of 10.9%) and maximum values in spring (average of 19.2%) (Fig. 2). Moisture decreased with soil depth across all studied soils (F= 36.89, $p < 0.0001$). Differences in soil moisture between the forest sites were only found in the spring ($p < 0.0001$) when soil in the Woodland site had a higher gravimetric water content than in the Forest. In each site, soil moisture values were similar for all vegetation covers in each season. Soil texture varied significantly between the forest sites with clayey soils in the Woodland (≈ 30% clay, 49% sand) and sandy soils in the

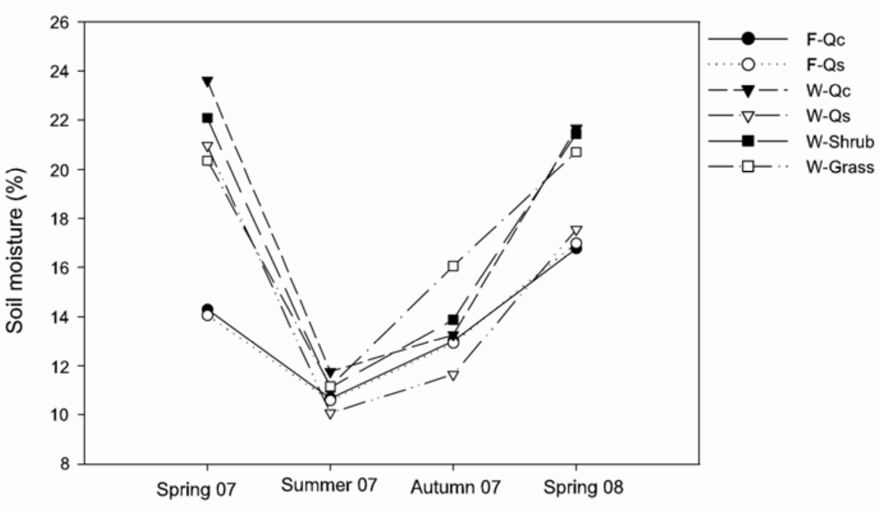

Figure 2. Soil water content estimated gravimetrically at the two forest sites (F-Forest; W-Woodland) and the four studied vegetation cover types (Qc- *Q. canariensis*; Qs- *Q. suber*; S-shrub; G- grass) at each sampling season.

Forest (≈ 16% clay, 65% sand). These differences affect the water holding capacity and moisture availability of their soils. Thus, under similar water content, sandy soils would proportionally have more water held at available potentials than clayey soils.

Soil organic matter (SOM) estimated as loss on ignition averaged over all samples 10.5% and varied from 0.77% to 24.93% in the Woodland and from 4.11% to 24.03% in the Forest (Appendix 1). Surface soil contained higher SOM (average of 12.7%) than the deeper layer (average of 8.35%) in all studied soils (F= 71.42, $p < 0.0001$). In both sites higher values of SOM were found in the soils beneath *Q. canariensis* and shrub than in the soils under *Q. suber* and herbaceous cover. Maximum litter biomass values (1.69 kg m^{-2}) were recorded in the Forest. Gaps with grass cover (in the Woodland site) had a significantly lower litter mass (0.09 kg m^{-2}) and litter layer thickness (0.56 cm) than any other microsite ($p < 0.0001$).

Soil ammonium averaged 7.3 µg g^{-1} in the Woodland and 4.0 µg g^{-1} in the Forest. Similar available phosphorus values were recorded in all soils (average of 2.5 µg g^{-1}). In general the concentration of both nutrients decreased with soil depth ($p<0.022$) except in the case of ammonium in the Forest ($p < 0.850$).

Soil total C and N ranged from 1.0% to 7.7% and from 0.1% to 0.7% respectively. Both soil total C and N decreased with soil depth ($p < 0.0001$) and total N content varied among soils. The Ct/Nt ratio averaged 14.7 and the ratio increased with soil depth (F= 43.75, $p < 0.0001$). The highest Ct/Nt values were recorded in *Q. suber* soils (>15.5) while soils beneath *Q. canariensis* in the

Table 2. Microbial P, C and N (mean and standard error) and their ratios. Seasonal and soil depth values are averaged for forest sites (Woodland and Forest), and canopy cover type (under *Q. canariensis*, *Q. suber*, shrub and grass).

	Pmic (µg g^{-1})	Cmic (µg g^{-1})	Nmic (µg g^{-1})	Cm/Nm	Cm/Pm	Nm/Pm	Cm/Ct (%)	Nm/Nt (%)	Pm/Pt (%)
Woodland									
Q. canariensis	25.67 (2.03)	1119.62 (70.32)	147.68 (10.85)	9.60	62.29	6.88	2.93	4.13	6.8
Q. suber	22.46 (2.03)	825.87 (48.72)	108.31 (8.32)	10.17	63.48	6.58	2.29	4.18	7.9
Shrub	21.32 (1.86)	966.27 (53.81)	138.58 (10.21)	8.97	79.61	8.97	2.50	4.11	7.0
Grass	12.18 (0.88)	772.75 (38.87)	107.86 (6.11)	7.57	93.80	12.19	2.11	4.14	4.4
Forest									
Q. canariensis	16.88 (1.38)	596.81 (32.39)	95.91 (5.55)	6.98	77.43	10.37	1.71	3.70	6.2
Q. suber	16.26 (1.43)	638.29 (37.64)	89.44 (4.99)	8.03	92.26	11.06	1.67	3.94	6.3
Woodland	20.39 (0.92)	921.13 (27.98)	125.61 (4.62)	9.08	74.81	8.66	2.46	4.14	6.5
Forest	16.57 (0.99)	617.55 (24.80)	92.68 (3.73)	7.51	84.85	10.71	1.69	3.82	6.2
All average	19.14 (0.70)	819.93 (21.41)	114.63 (3.39)	8.56	78.12	9.34	2.20	4.03	6.4

Woodland had the lowest carbon to nitrogen ratio (12.9). Total P in the soil ranged from 119 to 484 µg g^{-1} and average values were higher in the Woodland (312 µg g^{-1}) than in the Forest (259 µg g^{-1}). In the Woodland total P (F= 24.941, $p < 0.0001$) varied significantly among canopy cover types, with maximum average values (for two soil depths) beneath *Q. canariensis* (368 µg g^{-1}) and minimum in the grasslands (282 µg g^{-1}).

Microbial C, N and P pools

Microbial C pool (Cm) averaged over all samples 820 µg g^{-1} and ranged from 121 µg g^{-1} to 3232 µg g^{-1} (Table 2). The proportion of microbial C to total soil C averaged 2.2%. Microbial N (Nm) averaged 115 µg g^{-1} and contributed 4.0 % (range of 0.7% - 9.9%) to the total soil nitrogen while inorganic N (NH_4^+ and NO_3^-) contribution averaged 0.3%. Microbial P (Pm) in soils was, in average, 19 µg g^{-1} and, in the surface soil, it comprised about 9% of the total soil P. The proportion of inorganic available P to the total soil varied from 0.04% to 3.5% (mean of 0.89%). Average microbial nutrient ratios recorded were Cm/Nm: 8.6, Cm/Pm: 78.1 and Nm/Pm: 9.3. Microbial C, N and P values were positively and significantly correlated with soil moisture ($r > 0.46$, $p < 0.0001$) and soil organic matter ($r > 0.62$, $p < 0.0001$).

Microbial response to seasonal conditions

Soil microbial biomass did not significantly differ among seasons (Table 3, Fig. 3). Microbial carbon pool was significantly higher in the Woodland, where it ranged from 253 µg g^{-1} to 3232 µg g^{-1} and contributed 2.15 % to total soil C, than in the Forest where it varied from 1201 µg g^{-1} to 1772 µg g^{-1} and amounted 1.69% of total soil C ($p < 0.0001$). This difference was consistent across all seasons.

Table 3. Repeated measures ANOVA for microbial C, N and P of surface soil (0-8 cm) and subsurface soil (8-16 cm) from the two studied forest sites measured across four seasons. F and p-values for between effects (forest site and canopy cover type), within effect (season) and two way interactions are presented. Significant univariate results for each sampling season are indicated as *(0.05>p≥0.01); **(0.01>p≥0.001); ***(p<0.001).

	Cm						Nm						Pm					
	F	p	Sp07	Su07	A07	Sp08	F	p	Sp07	Su07	A07	Sp08	F	p	Sp07	Su07	A07	Sp08
Surface soil																		
Forest site	40.45	0.000	**	**	***	***	15.55	0.000	**	**		***	18.127	0.000	***		*	***
Season	0.99	0.399					36.82	0.000					32.455	0.000				
Season* Forest site	6.56	0.000					10.74	0.000					24.233	0.000				
Vegetation cover	4.21	0.042	**				10.74	0.001	**			**	0.552	0.459				
Forest site* Vegetation cover	13.74	0.000	**			***	3.79	0.053	*				0.503	0.479				
Subsurface soil																		
Forest site	17.25	0.000	*		*	**	1.000	0.324	***		***	**	11.500	0.002	***		***	
Season	2.05	0.110					34.594	0.000					22.780	0.000				
Season* Forest site	1.88	0.137					28.598	0.000					22.646	0.000				
Vegetation cover	3.99	0.048					3.158	0.078	*				3.051	0.083				
Forest site* Vegetation cover	2.77	0.098	*				1.434	0.233					0.149	0.700				

Forest site: Woodland and Forest

Vegetation cover type: *Q. canariensis* and *Q. suber*

Season: spring 07, summer 07, autumn 07, spring 08

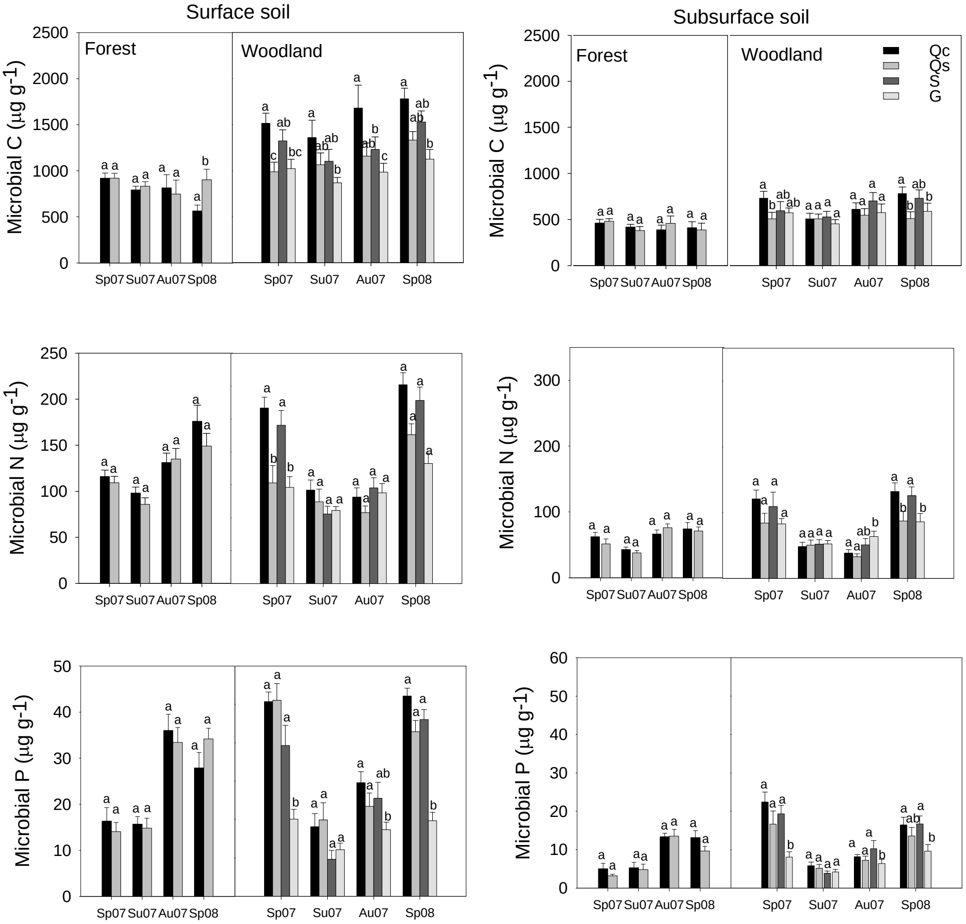

Figure 3. Microbial C, N and P estimated in the soils under the different types of vegetation cover (*Q. canariensis* (Qc), *Q. suber* (Qs), shrub (S) and grass cover (G)) in the two studied forest sites (Forest and Woodland) across the four sampling seasons (Spring 07, Summer 07, Autumn 07, Spring 08). Data is presented for the two soil depths, surface soil (0-8 cm) and subsurface soil (8-16 cm). Letters indicate differences between groups for each season ($p < 0.05$ after FDR corrections). Bars represent standard error of the mean.

Microbial N and P pools varied seasonally with maximum values in spring and minimum values in summer (although the seasonal patterns differed between forest sites). The most important seasonal variations were observed for microbial P which values changed twofold from the spring (~24 µg g $^{-1}$) to the summer (10 µg g $^{-1}$). On average, soils in the Woodland had higher microbial N (126 µg g $^{-1}$) and microbial P (20 µg g $^{-1}$) than soils in the Forest (N: 93 µg g $^{-1}$, P: 17 µg g $^{-1}$) although this pattern was reversed in the autumn when Pm pool was larger in the Forest. Summer Nm and Pm values were similar for both forest sites while the largest differences between sites were recorded during the spring ($p < 0.003$), when the Woodland presented the highest values.

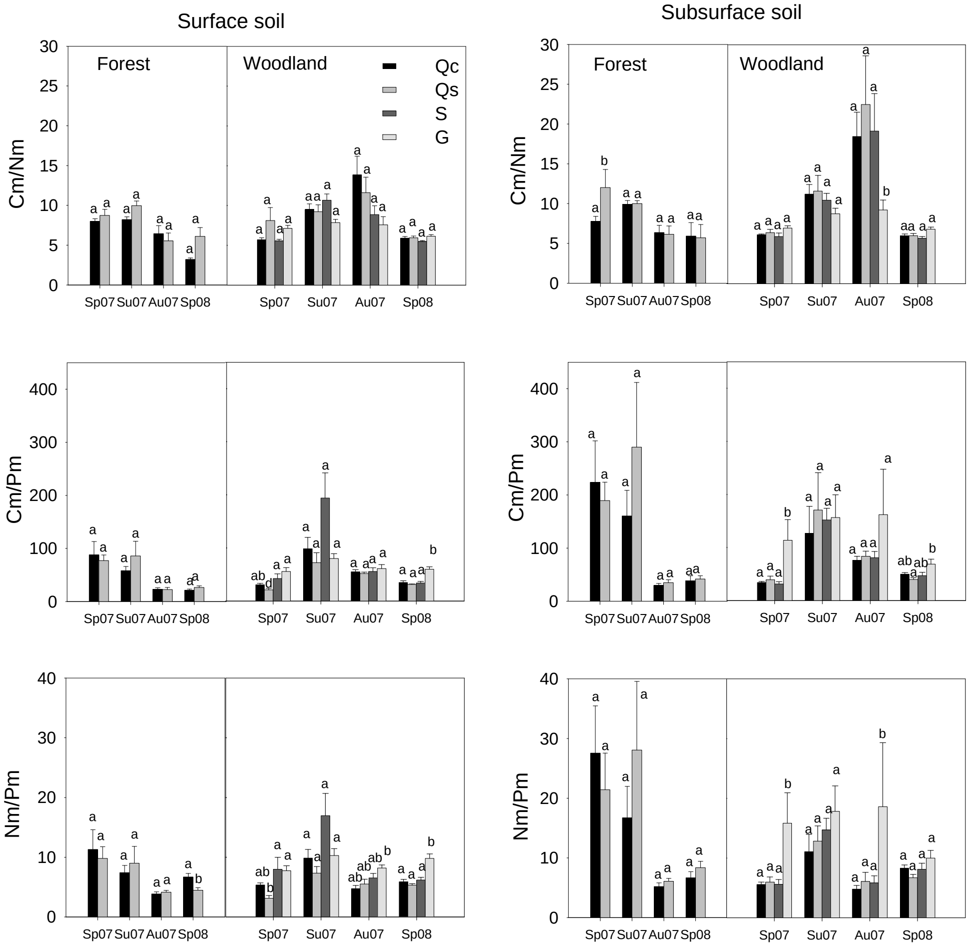

Figure 4. Microbial ratios -Cm/Nm, Cm/Pm and Nm/Pm- for each season (Spring 07, Summer 07, Autumn 07, Spring 08), and vegetation cover type (*Q. canariensis* (Qc), *Q. suber* (Qs), shrub (S) and grass cover (G)) in the two forest sites (Forest and Woodland). Data is presented for the two soil depths (0-8cm and 8-16cm). Letters indicate differences between groups for each season ($p<0.05$ after FDR corrections). Bars represent standard error of the mean.

Microbial ratios (Cm/Nm; Cm/Pm; Nm/Pm) changed seasonally ($p<0.0001$) and generally differed between the two forest sites (except for the summer), due to their distinct values of Nm and Pm (Fig. 3 and 4).

Season also affected the non microbial pools of available nutrients (Fig. 5). K_2SO_4-extractable dissolved organic carbon values increased in summer matching up with a slight but not significant decrease in Cm, and declined in spring when the microbial C values were higher. K_2SO_4-extractable dissolved organic nitrogen, inorganic N (ammonia and nitrate) and available inorganic P

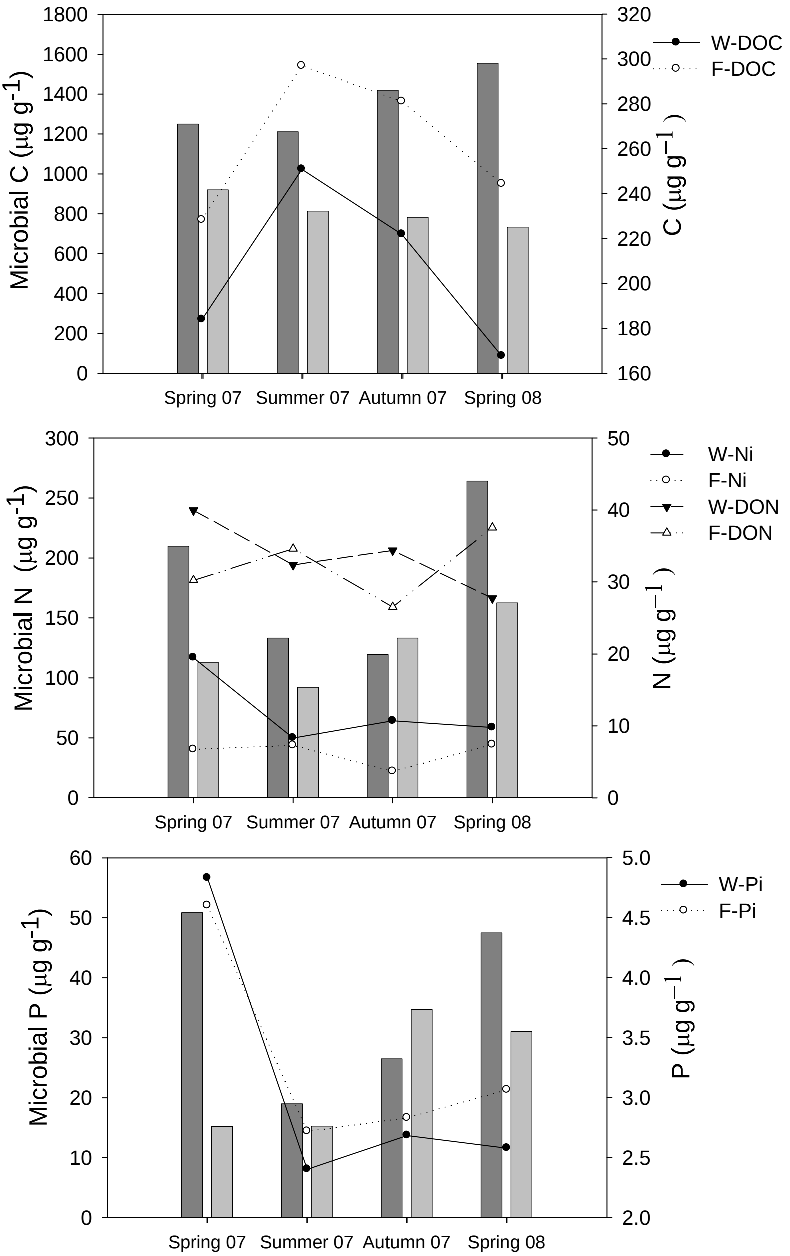

Figure 5. Microbial C, N and P, K_2SO_4-extractable dissolved organic carbon (DOC) and nitrogen (DON), inorganic nitrogen (extractable ammonium and nitrate; Ni) and available inorganic phosphorus (Pi) estimated in the surface soil (0-8 cm) beneath *Quercus* cover in the Woodland and the Forest site across the sampling seasons.

declined in summer and autumn and increased in spring, mirroring the pattern observed for Nm and Pm ($r \approx 0.35$, $p < 0.0001$).

Effect of vegetation cover type on soil microbial biomass

In the Forest site there were no significant differences in microbial C, N, P pools and their ratios between the soils of the two Quercus species at any sampling time and soil depth (Fig. 3 and 4).

In the Woodland the effect of the vegetation cover type varied across seasons: significant differences among cover types were observed in the spring while similar microbial values were recorded for all vegetation types in summer. The effect of cover type on soil microbial pools was larger in the upper soil layer ($p < 0.002$ for Cm, Nm and Pm) than in the subsurface soil ($p < 0.029$ for Pm). Higher values of microbial C were recorded in *Q. canariensis* and shrub soils compared to those estimated in soils beneath *Q. suber* and the least beneath grass cover in forest gaps, which constantly showed the lowest Cm values and were not affected by seasonality. As occurred with Cm, soils of *Q. canariensis* and shrubs had higher microbial N than soils under *Q. suber* and herbaceous cover. In contrast microbial P was similarly high beneath the shrubs and the two oak trees while minimum values were consistently recorded in the soils under grass cover. The largest changes in Cm/Nm among vegetation cover types were observed in autumn when the ratio decreased from the two Quercus species to shrub soils and grass soils.

Cm/Pm and Nm/Pm values tended to vary seasonally and the highest values were recorded in summer related to the limited amount of microbial P. Soils beneath grass cover, in contrast to other cover types, showed no similar Cm/Pm and Nm/Pm values across seasons.

Microbial properties and soil depth

All microbial C, N and P decreased twofold with soil depth ($p < 0.0001$); in particular carbon from 1106 μg g^{-1} in the upper soil to 534 μg g^{-1} in the subsurface soil, nitrogen from 158 μg g^{-1} to 71 μg g^{-1}, and phosphorus from 28 μg g^{-1} to 10 μg g^{-1}. Cm/Nm was higher in the subsurface soil, although thisdifference was only significant in autumn, when values increased from 8.9 to 13.6. Cm/Pm and Nm/Pm ratios were always significantly higher in the subsurface soil (104.2 and 10.3) than in the upper soil (64.0 and 5.2 respectively) ($p < 0.0001$).

DISCUSSION

Soil microbial carbon, nitrogen and phosphorus in Mediterranean forests

Microbial C and N averaged 820 μg g^{-1} and 115 μg g^{-1} in the studied Mediterranean forest soils. These values fell within the range of Cm and Nm estimates presented by Wardle (1992) for tropical (Cm: 653-986 μg g^{-1}; Nm: 65-100 μg g^{-1}) and temperate forests (Cm: 736-877 μg g^{-1}; Nm: 93 μg g^{-1}).

They were also in accordance with the few examples of Mediterranean forests: Cm range of 975-1601 µg g $^{-1}$ in a pine forest (Hernández et al., 1997) and Nm of 72-178 µg g $^{-1}$ in an oak savanna (Gallardo et al., 2000).

Recently Cleveland and Liptzin (2007) have revealed the existence of a C:N:P Redfield- like ratio for the soil microbial biomass at the global scale (60:7:1) and for forest soils (74:9:1) that is very close to the average microbial ratio that we have found (78:9:1). Microbial stoichiometry relations are partly dependent on the validity of the microbial C, N and P estimates which, in fumigation procedures, are dependent on the k-factors used (Ross et al., 1996). Our results confirm that microbial biomass stoichiometry is well constrained and considering that the use of laboratory-determined fixed factors may not be accurate for a diverse soil community and could mask differences between soil types and depths, microbial N:P could be cautiously used, in addition to N:P ratios in plants and soils, to estimate nutrient deficiency in terrestrial ecosystems (Cleveland and Liptzin, 2007).

The microbial P recorded in the studied soils (19 µg g $^{-1}$) was close to the lowest Pm values recorded by Joergensen (1995) in 38 beech forest soils (18-174 µg g $^{-1}$). However the proportion of total P immobilized by microorganisms in the surface soil (8.8%) was higher that that of C (2.7%) and N (4.7%) what suggests that microbial biomass may play an important role in regulating plant phosphorous supply in the studied forests (Joergensen et al., 1995; Jonasson et al., 1996). The microbial fraction of the total N and P pool was almost ten times higher than the estimated available inorganic N (NH_4^+ and NO_3^-; 0.38% of the total N) and available P (0.89% of total P). This could implicate that nutrient availability for plants might be strongly controlled by microbial dynamics: a flush of available N and P for plants could be released after microbial population decline while microbial population growth might be associated with a strong competition for these growth-limiting resources (Lipson et al., 1999; Schmidt et al., 2007).

Seasonal variations of microbial C, N and P

Microbial C, N and P in the studied soils showed distinct seasonal patterns that were reflected in changes in the microbial component ratios. Seasonal variations of soil microbial carbon had been registered in many ecosystems (Díaz-Raviña et al., 1993; Bohlen et al., 2001; Miller et al., 2009) including Mediterranean and semi-arid zones (Goberna et al., 2007; Mlambo et al., 2007). Seasonality seems to affect microbial populations through changes in soil moisture and temperature, but it may also indirectly modulate substrate availability through plant phenology (Rinnan et al., 2008).

In this mediterranean-type climate we expected a decline in microbial carbon during the summer as a result of drought stress. However, microbial C showed no significant differences across seasons, although values tended to be lower during the summer time and they were correlated with soil moisture content. Several processes can occur during summer drought that would explain these results. Under low moisture conditions, soil microorganisms may become isolated in a landscape of disconnected water pockets that impedes the diffusion of substrate, limit microbial growth and may cause death by starvation (Killham et al., 1993; Xiang et al., 2008). On the other hand this disconnection also prevents microorganism to be predated by soil fauna (protozoa, amoeba) which activity and mobility are reduced by soil drought (Kuikman et al., 1989).

Drought stress also affects microbial physiology. Low water potential induces microbial dehydration and might eventually cause death. Drought-tolerant microbes can be inherently protected against low moisture (e.g. thicker cell walls of gram-possitive bacteria) or have the capacity to adapt to the external low water availability by accumulating osmolites in their cytoplasm) (Schimel et al., 2007). As a result of drought stress up to 30% of carbon resources can be bound in cytoplasmic osmotic protection molecules, what negatively affects microbial activity and population growth (Schimel et al., 1989). In this study microbial C was estimated using the fumigation-extraction technique that recovers only a fraction of the total microbial C, most of which is cytoplasmic, and relates it to the total by an empirical constant (K_c). Changes in the concentration of cytoplasmic C may therefore be a source of error in microbial biomass measurements (Schimel et al., 1989; Ross et al., 1996). We suggest that in our studied soils a fraction of the total microbial population might have died during summer drought, what together with summer root decay (Joergensen et al., 1994) could account for the K_2SO_4-extractable DOC peak recorded in this season. The estimated Cm values may instead reflect the increased cytoplasmic concentrations resulting from the physiological survival strategy of the resistant microbial fraction.

In contrast to Cm, a large decrease of microbial N and P was recorded during summer that supported our hypothesis. Cytoplasmic osmolytes used by bacteria to withstand drought stress are amino compounds (Csonka, 1989), while fungi use polyols that do not contain N nor P (Witteveen and Visser, 1995). We can speculate whether the observed decrease in Nm and the change in the microbial C/N ratio could indicate that during the summer drought microbial community composition shifted to a higher abundance of fungi (Schimel et al., 2007).

Increased soil microbial N and P were observed during the wet seasons (spring and autumn). In a similar seasonal study, Nielsen et al. (2009) observed that the higher water content increased the accessibility of nutrients and enhanced microbial growth, and consequently N and P immobilization in the microbial biomass. In addition root exudates, root decay and fresh litter shed by the winter-deciduous *Q. canariensis* could constitute an important input of easily decomposable organic substrate for soil microorganism growth (Gallardo and Merino, 1993; Joergensen et al., 1994). Both mechanisms could account for the increased microbial nutrient immobilization.

There were also seasonal changes in the concentration of available inorganic nutrients that could be related to variations in temperature, moisture and quality of organic matter, which control microbial processes such as mineralization and immobilization (Gallardo and Merino, 1992; Schmidt et al., 1999). Despite the differences in soil water availability in the two forest sites due to distinct soil texture and water holding capacity, common patterns were found for inorganic nutrients dynamics: High levels of available N and P, probably resulting from net mineralization and liberation, were recorded during Spring 2007 when conditions were favorable for microbial growth and activity (Fig. 2). During summer Nm and Pm decreased, but there is no evidence of increasing available nutrients thus it is possible that inorganic nutrients resulted from microorganisms decay may have been taken up by plants. In Autumn and Spring 2008, N and P were immobilized in the growing microbial biomass. From the synchrony between the available nutrients temporal patterns and microbial biomass dynamics we could infer that in these forests microbes might not be competing with plants for soil resources, but instead could be covering plants nutrient demand. However this hypothesis remains to be critically tested.

Microbial biomass is related to forest site conditions

In a previous study carried out in the same forest sites Quilchano and Marañón (2002) observed lower enzymatic (dehydrogenase) activity in the Forest site. In accordance, our results indicated that microbial C was higher in the Woodland, particularly in soils of *Q. canariensis*, than in the Forest. This difference was consistent across seasons suggesting that the driving mechanism of this variation was not affected by seasonal changes in the environmental conditions. Both sites differ in several physiographical aspects that could contribute to their distinct soil microbial properties; however we suggest that the higher clay content in the Woodland soils may be among the most important factors accounting for this difference since clay has a higher capacity to adsorb nutrients and organic matter. It also reduces decomposition rates, buffers pH changes, provides microorganisms shelter against microbivores and increases the soil water holding capacity, all of which promote microbial biomass growth (Oades, 1988; Van Veen and Kuikman, 1990; Wardle, 1992).

Season determines canopy cover type effect on microbial biomass

Canopy cover type accounted for main differences in soil microbial parameters within the oak forests; although those patterns were only apparent under favorable environmental conditions, once the main constraining factor (water stress) disappeared. The influence of seasonal conditions on the effect of vegetation on microbial C, N and P has been also detected in other studies (Goberna et al., 2007; Malchair and Carnol, 2009). In addition, the effect of canopy cover type was mostly observed in the upper soil layer, while conditions were more homogeneous in the subsurface soil suggesting a plant cover effect on soil probably through differences in litter fall quantity and quality (Augusto et al., 2002). Billore et al. (1995) found a strong positive correlation between microbial and root biomasses, thus vegetation could have also controlled soil microbial processes through differences in the root systems. Nevertheless, not all the variability observed among microhabitats should be attributed the canopy cover type but to other belowground characteristics (e.g. soil depth) that could in turn be the underlying reason for the distinct cover type.

Soils underneath *Q. canariensis* and shrub sustained a higher microbial C and microbial N than soils beneath *Q. suber*. Differences in the litter quality among the vegetation types (lower N content and higher C/N ratio in *Q. suber*; (Aponte et al., 2010)) would have induced a distinct soil organic matter content, total soil nitrogen and C/N ratio and subsequently affected soil microbial C and N (Smolander and Kitunen, 2002; Kara et al., 2008; Rinnan et al., 2008).Soils in the forest gaps had the lowest microbial values compared to the other studied microsites. These soils had a higher proportion of nitrogen (Cm/Nm: 7.6) but a lower fraction of phosphorus (Cm/Pm: 93.8), which could be interpreted as a P limitation (Cleveland and Liptzin, 2007). The lack of canopy cover in the forest gaps reduced litter input (0.09 kg/m^2). The small inputs of P could constrain microbial growth and activity (García et al., 2002; Goberna et al., 2007) and may also increase exposure of this soil to rapid shifts in soil temperature and moisture. In contrast to the Woodland, no differences were found in the microbial components between the soils beneath the two *Quercus* species in the Forest. The vegetation structure of this site, where oak trees had a higher density (Table 1) and formed a closed canopy, promoted higher homogeneity of the litter layer that was reflected in the soil chemical and microbiological properties (Quilchano et al., 2008).

Microbial properties and soil depth

In the studied forest sites all measured microbial constituents, as well as most of the studied soil variables which may influence the microbial pool (soil moisture, SOM, total N, C and P), where higher in the surface soil than in the subsurface soil. A similar pattern has been reported by other authors who also detected a decline in microbial activity with soil depth (Ross et al., 1996). Two main mechanisms could be driving this pattern: first a decrease in the quality and quantity of substrate; the subsurface soil contained less organic matter and probably had a higher fraction of recalcitrant compounds resulted from an advanced decomposition (Gaudinski et al., 2000). Second, the lower moisture content of the subsurface soil could impede the diffusion of the scarce substrate thought the disconnected water pockets and limit its supply to the isolated microbial populations (Xiang et al., 2008). Both mechanisms may act simultaneously inducing microbial starvation and limiting microbial population growth in the subsurface soil (Fontaine et al., 2007).

Microbial ratios increased with soil depth in our soils. Contradictory patterns of change in Cm/Nm have been found in other studies. For example Ross et al. (1996) observed that Cm/Nm declined with depth in forest soils, whereas Raubuch and Joergensen (1996) detected only a small difference in the microbial C-to-N ratios in the organic layer (6.0) and in mineral soil (7.1). Variations in the C/N ratio are commonly related to shifts in microbial community composition (bacteria vs. fungi), since the fungi have higher carbon: element ratios (C:N: 5-17; N:P=15) than the bacteria (C:N: 6.5 N:P =7) (Cleveland and Liptzin, 2007). In his work Fierer et al. (2003) observed that decreasing substrate and moisture availability along the soil depth profile induced changes in microbial community composition and lead to a community dominated by drought and starvation-tolerant organisms.

CONCLUSIONS

- In the studied Mediterranean forests soil microbial biomass was affected by season, vegetation cover type and structure, and soil depth.

- Seasonal changes in microbial nutrient content were observed for N and P, which had higher values during the wet seasons (spring and autumn), unlike microbial C.

- Differences in the soil microbial properties between forest sites or among canopy cover types were found in spring but not in summer.

-Microbial C, N and P significantly decreased from surface to subsurface soil, in every season and forest site

The conjoint study of the effects of season, vegetation cover type and structure and soil depth on microbial biomass in two forest sites has shown the existence of relevant seasonal interactions between most of these factors. The typical seasonal pattern of Mediterranean climate strongly determines the soil moisture regime, affects microbial growth and conditions the influence of other biotic factors on microbial biomass, playing an important role in the nutrient release-immobilization cycles and in the nutrient availability for plants in these forests.

Overall, this study provides valuable information on soil microbial seasonal dynamics in Mediterranean forests that may contribute to enhance our understanding on how climate change could affect to microbial control on nutrient availability.

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APPENDIX

Appendix 1. Characteristics of the surface (0-8 cm) and subsurface (8-16 cm) soil beneath the studied vegetation cover types in the two forest sites. Data represent mean values and standard error. Letters indicate differences between groups p<0.05 after fdr correction.

	Organic Matter(1) (%)	Total C (%)	Total N (%)	Total P (ppm)	Ct/Nt	NH_4 –N (ppm)	NO_3 –N (ppm)	Available P (ppm)	Clay(2) (%)	Sand(2) (%)	Litter mass(3) (kg m^{-2})	Litter thickness(3) (cm)
Superficial soil												
Woodland												
Q. canariensis	14.93 (0.51) a	4.36 (0.19) a	0.43 (0.02) a	411.9 (9.5) a	10.67 (0.50) a	9.87 (1.24) a	2.56 (0.28) a	3.17 (0.31) ac	32.7 (2.1) a	46.4 (2.5) a	0.87 (0.07) ab	4.25 (0.26) ab
Q. suber	12.39 (0.37) bc	4.42 (0.18) a	0.33 (0.01) b	332.6 (8.5) b	13.84 (0.43) b	9.05 (0.76) a	2.66 (0.27) a	3.07 (0.27) ac	24.8 (2.4) ab	55.2 (4.1) ab	1.05 (0.15) b	2.95 (0.40) c
Shrub	14.15 (0.58) ab	4.37 (0.18) a	0.40 (0.02) a	322.1 (10.1) b	12.04 (0.66) ac	9.09 (0.85) a	2.95 (0.30) a	2.56 (0.20) ab	30.1 (3.3) a	46 (4.7) a	0.63 (0.04) a	5 (0.44) a
Grass	11.40 (0.50) c	4.12 (0.13) a	0.31 (0.01) bc	282.0 (9.3) c	14.11 (0.45) bd	5.7 (0.60) b	2.6 (0.28) a	2.31 (0.24) b	30.8 (3.5) a	47.4 (5.0) a	0.04 (0.01) c	0.5 (0.18) d
Forest												
Q. canariensis	12.51 (0.79) c	3.95 (0.20) a	0.32 (0.02) bc	297.8 (10.4) bc	13.44 (0.70) bc	4.79 (0.38) b	2.05 (0.13) a	3.68 (0.24) ac	13.1 (1.3) bc	67.8 (1.5) bc	1.69 (0.10) d	4.5 (0.27) ab
Q. suber	10.81 (0.44) c	4.28 (0.14) a	0.28 (0.01) c	299.1 (8.9) bc	16.02 (0.51) bd	4.26 (0.29) b	1.46 (0.14) b	2.92 (0.17) ac	19.2 (2.2) c	63.4 (2.5) c	1.69 (0.13) d	3.53 (0.33) bc
Subsuperficial soil												
Woodland												
Q. canariensis	9.17 (0.37) a	3.47 (0.13) a	0.24 (0.01) a	324.4 (11.4) a	15.09 (0.33) a	5.72 (0.73) a	2.02 (0.27) ab	2.03 (0.14) a	na	na	-	-
Q. suber	7.08 (0.35) b	2.87 (0.11) b	0.17 (0.01) b	228.2 (11.9) b	17.19 (0.38) a	5.8 (0.51) a	1.73 (0.21) ac	1.98 (0.14) a	na	na	-	-
Shrub	9.32 (0.44) a	3.57 (0.16) a	0.25 (0.01) a	317.3 (13.3) a	15.34 (0.52) a	6.62 (0.71) a	2.13 (0.22) a	1.46 (0.12) b	na	na	-	-
Grass	8.32 (0.29) a	3.13 (0.11) ab	0.21 (0.01) ac	281.1 (8.6) c	15.44 (0.48) a	6.01 (0.50) a	2.54 (0.28) a	1.82 (0.18) ab	na	na	-	-
Forest												
Q. canariensis	8.94 (0.66) a	3.24 (0.17) ab	0.22 (0.01) a	230.7 (8.7) b	15.63 (0.71) a	3.58 (0.40) b	1.35 (0.22) bd	2.64 (0.21) c	na	na	-	-
Q. suber	7.30 (0.29) b	3.05 (0.11) ab	0.19 (0.01) bc	209.4 (8.1) b	17.03 (0.42) a	3.41 (0.36) b	1.17 (0.14) cd	1.93 (0.13) a	na	na	-	-
All average	10.53 (0.18)	3.73 (0.05)	0.28 (0.01)	294.7 (3.7)	14.7 (0.2)	7.41 (0.36)	2.1 (0.07)	2.47 (0.06)	25.1 (1.4)	54.35 (1.8)	1.29 (0.08)	3.76 (0.18)

(1) OM meassured by loss on ignition

(2) Textural variables were determined only for 0-25cm soil depth, na mean non available data

(3) Litter variables were measured on the surface of each sample point

Capítulo 7.

Efectos indirectos de la especie huésped en la comunidad de hongos ectomicorrícicos

Este capítulo reproduce el siguiente manuscrito:

Aponte, C., García, L.V., Marañón, T., Gardes, M. 2010. *Indirect effects of host species on ectomycorrhizal fungi: Leaf fall quality induces changes in fungal communities on the roots of co-occurring Mediterranean oaks*. Soil Biology and Biochemistry 42, 788-796.

Capítulo 7. **Efectos indirectos de la especie huésped en la comunidad de hongos ectomicorrícicos**

RESUMEN

Las especies arbóreas pueden modificar las condiciones abióticas del suelo a través de la distinta calidad de su hojarasca, lo que a su vez puede influir en la composición de la comunidad de hongos ectomicorrícicos asociados a sus raíces. En este trabajo se investigó la importancia de este efecto indirecto empleando un análisis de modelos causales. Se identificaron hongos ectomicorrícicos en las raíces de dos quercíneas coexistentes presentes en los bosques del sur de España, *Q. suber* (perenne) y *Q. canariensis* (marcescente), utilizando métodos moleculares basados en PCR. También se analizaron químicamente el desfronde, la hojarasca y el suelo muestreados bajo la copa de los árboles estudiados para determinar las concentraciones de nutrientes clave.

En total se identificaron 69 unidades taxonómicas operacionales (OTUs). *Tomentella* y *Russula* fueros los géneros más frecuentes y abundantes y con un mayor número de especies. Los hongos con carpóforos epigeos y resupinados formaron el 60% y 34% de las micorrizas identificadas, respectivamente. La distribución de especies de ectomicorrizas estuvo significativamente relacionada con contenido en calcio de la hojarasca, que fue mayor bajo la especie marcescente debido a diferencias en la calidad del desfronde.

La evaluación de distintos modelos causales alternativos mediante el método d-sep reveló que sólo aquellos modelos que consideraban el efecto indirecto de las especies en el suelo mediado por la calidad del desfronde y la hojarasca se ajustaban significativamente a la covariación de patrones observados entre la especie huésped, las condiciones ambientales (hojarasca y suelo) y la comunidad de hongos ectomicorrícicos.

Indirect host effect on ectomycorrhizal fungi: Leaf fall and litter quality explain changes in fungal communities on the roots of co-occurring Mediterranean oaks

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Abstract

Host trees can modify their soil abiotic conditions through their leaf fall quality which in turn may influence the ectomycorrhizal (ECM) fungal community composition. We investigated this indirect interaction using a causal modeling approach. We identified ECM fungi on the roots of two coexisting oak species growing in two forests in southern Spain - Quercus suber (evergreen) and Q. canariensis (winter deciduous)- using a PCR-based molecular method. We also analysed the leaf fall, litter and soil sampled beneath the tree canopies to determine the concentrations of key nutrients. The total mycorrhizal pool was comprised of 69 operational taxonomic units (OTUs). Tomentella and Russula were the most species-rich, frequent and abundant genera. ECM fungi with epigeous and resupinate fruiting bodies were found in 60% and 34% of the identified mycorrhizas, respectively. The calcium content of litter, which was significantly higher beneath the winter-deciduous oak species due to differences in leaf fall quality, was the most important variable for explaining ECM species distribution. The evaluation of alternative causal models by the d-sep method revealed that only those considering indirect leaf fall-mediated host effects statistically matched the observed covariation patterns between host, environment (litter, topsoil, subsoil) and fungal community variables.

***Keywords**: Indirect host effect, Mediterranean forest, mycorrhiza, path analysis, Quercus suber (cork oak), Quercus canariensis (Algerian oak), resupinate fungi, tree-soil-fungi interactions.*

INTRODUCTION

Mycorrhizal symbioses are essential for oak trees for acquiring nutrients under natural conditions (Smith and Read, 1997). Ectomycorrhizal (ECM) fungi supply plants with water and nutrients by increasing their foraging area and absorbing efficiency, and they provide an ample range of other beneficial effects as well, in exchange for photosynthesized products (Schutzendubel and Polle, 2002; Frey-Klett et al., 2005; Egerton-Warburton et al., 2007; Finlay, 2008). ECM communities contain a high diversity of fungal taxa (Taylor and Alexander, 2005), which are associated with a variety of functional strategies that contribute to forest ecosystem stability and functioning (Perry et al., 1989; Nara, 2006).

During the last decade, numerous studies have tried to unveil the role that natural factors, such as environmental conditions and host plant community composition, have on the assemblage of ECM communities (Conn and Dighton, 2000; Dickie and Reich, 2005; Buée et al., 2007). Soil abiotic conditions, namely, soil moisture, pH and nutrient availability, influence the performance and assemblage of fungal species (Brearley, 2006; Cavender-Bares et al., 2009). Host trees may directly affect the assemblage of their mycorrhizal community by exerting a selection for mycorrhizal species (Ishida et al., 2007; Tedersoo et al., 2008).

In a recent study Morris et al. (2008) studied the separate effects of soil conditions and host tree species on the composition of ECM communities in a California mixed oak forest and concluded that both explained a significant proportion of the variation in ECM species distribution. These effects have usually been investigated independently despite the fact that host trees, acting as ecosystem engineers, may also indirectly shape ECM communities through their ability to modify the abiotic conditions of their environment (Jones et al., 1994; Bennett et al., 2009). The magnitude and direction of these changes are species-specific, and they can be mediated by litter quality and biomass, root exudation and nutrient uptake (Gobran et al., 1998; Mitchell et al., 2007). The complex interactions among host plants, environmental conditions and fungal communities are difficult to disentangle and the relative importance of the indirect host effects on this mutualistic relationship remains unclear.

Oak forests of southern Spain are an important economic and social resource at the same time that they are a hotspot of endemic and relict plant species (Médail and Quézel, 1999; Anonymous, 2005). Despite the well-known dependence of oaks on mycorrhizal fungi, only a limited number of studies have been done on the belowground ECM communities in oak forests (Walker et al., 2005; Buée et al., 2007; Avis et al., 2008), and even fewer have been carried out in areas with seasonally dry mediterranean-type climates (Richard et al., 2005; Smith et al., 2007; Morris et al., 2008). In this study we aimed to investigate the importance of the indirect effects of host tree species on the ECM community. For that purpose we studied the ECM fungal community on the roots of two coexisting oak trees, the sclerophyllous evergreen *Quercus suber* (cork oak) and the winter-deciduous *Q. canariensis* (Algerian oak), under the mediterranean-type climate of southern Spain, using PCR-based molecular methods.

The research objectives of this study were: i) to assess the diversity and structure of the ECM communities on the roots of these two co-occurring *Quercus* species in two Mediterranean mixed-oak forests, using DNA-based identification techniques; ii) to analyse the relationships between the distribution of ECM species and the measured litter and soil variables by using multivariate methods; iii) to evaluate the extent to which the composition and diversity of the ECM community may be explained by the soil conditions, the host identity and the litter-mediated changes in the top-soil environment (soil and litter) and iv) to compare among several alternative causal models explaining the plant-soil-fungal interactions, in order to test the hypothesis that the studied oak host species, through the differences in their leaf fall quality, may produce key changes in the litter and topsoil chemical composition that in turn, may affect the ECM community assemblage.

MATERIALS AND METHODS

Study area and forest sites

The study area is located in the Aljibe Mountains, in the south of Spain. The region has a rough topography, with the highest peak reaching 1092 m a.s.l. The bedrock dominated by Oligo-Miocene sandstone originates acidic, nutrient-poor soils (Palexeralfs; Soil Survey Staff, 2010) frequently interspersed with layers of marl sediments that yield soils richer in clay (Haploxererts; Soil Survey Staff, 2010). The climate is of the sub-humid mediterranean-type with most rainfall (95%) occurring from October to May. See detailed descriptions of the area in Ojeda et al. (2000) and Anonymous (2005)

The vegetation is dominated by evergreen cork oak (*Quercus suber* L.), mixed with the winter-deciduous Algerian oak (*Q. canariensis* Willd.), which is locally abundant in the valley bottoms (Urbieta et al., 2008). The arborescent shrubs *Phillyrea latifolia* L. and *Pistacia lentiscus* L. dominated the understorey. The area has been protected since 1989 as "Los Alcornocales" (meaning "the cork oak forests") Natural Park (Anonymous, 2005).

Two structurally different forest sites, 40 km apart, were selected within the study area. The site at San Carlos del Tiradero (36º 9’ 46’’ N; 5º 35’ 39’’ W), hereafter called "Forest", was located in the south of the Park near the coast at 335–360 m a.s.l. on a NE facing slope. The mean annual rainfall is 964 mm, and the mean annual air temperature is 16.6 ºC, with a minimum of 4.1 ºC. The Forest stand had a high density of trees (769 stems ha^{-1}) with a basal area of 47 m^2 ha^{-1}(estimated on trees with dbh $\geq$ 1.6 cm).

The other site, at La Sauceda (36º31'54''N; 5º34'29''W), hereafter called "Woodland", was located inland, in the north of the Park, at 530–560 m a.s.l. on a NW facing slope. It has a mean annual temperature of 15.5ºC, with a minimum of 1.8 ºC and a mean annual rainfall of 1470 mm. The Woodland tree density was relatively low with 219 stems ha^{-1} and a basal area of 22 m^2 ha^{-1} (Pérez-Ramos et al., 2008).

Sampling design

At each forest site (f: Forest, w: Woodland), six adult individuals of *Q. suber* (S) and six of *Q. canariensis* (C) located in a matrix of coexisting oak species and spread across approximately 1ha were selected. Thus a total of 24 oaks were sampled that can be grouped into four categories (Cf, Cw, Sf, Sw; each with six replicates) of two combined factors: oak species and forest site. The selected trees were estimated to be more than 50 years old.

Leaf fall, litter, topsoil (~1400 cm^3, 0-25 cm depth) and subsoil (~1400 cm^3, 25-50 cm depth) were sampled beneath the canopy of each selected oak at approximately 2 m from the trunk in November 2006. Annual leaf fall was collected by four traps (50 cm diameter) located under each tree. The contents were removed, and the leaves were separated and dried. Two 30x30 cm quadrats were sampled to assess the litter biomass, by the harvesting and drying method (expressed as kg dry mass m^{-2}). Both leaf fall and litter samples were composed by leaves from one oak species since the closest neighbours of the selected trees were individuals of the same species and they had no significant understorey cover under their canopy. Once the litter layer was removed, cores of soil were extracted with a cylindrical auger; four samples of topsoil (0-25 cm) and four of subsoil (25-50 cm) were taken under each oak tree in the four cardinal directions and pooled into single representative samples.

Superficial roots (~0-15 cm depth) approximately equal in length (≈20 cm) were taken from each selected tree, close to the litter and soil sampling points, in November 2007. Root samples were kept moist in sealed plastic bags and transported inside an ice-box to the laboratory, where they were stored at 4 ºC. Within two days, each root system was examined under a binocular microscope, and 20-22 ectomycorrhizal root tips from each tree were randomly picked free of debris, removed with tweezers and individually stored frozen in 100 µL of 2X CTAB buffer.

Leaf fall, litter and soil analyses

Samples of leaf fall and litter were dried at 70ºC, weighed, and ground for chemical analysis. Soil samples were dried (30 ºC for 48-72 hours) and crushed to pass through a 2 mm sieve. Soil acidity (pH) was determined potentiometrically in a 1:2.5 soil:H_2O solution. The percentage of soil carbon was estimated using a Total Organic Carbon Analyzer (TOC-Vesh). The available P was estimated using the Bray 1 method (Bray and Kurtz, 1945). The total concentration of several macro-nutrients (Ca, K, Mg, P and S) in plant tissues and soils were determined by acid digestion with nitric acid or aqua regia followed by ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry). Plant and soil nitrogen was determined by using Kjeldahl digestion and subsequent distillation–titration in a Bran-Luebbe Autoanalyzer. See methods in Allen (1989).

Ectomycorrhizal DNA extraction, amplification and sequencing

From each ectomycorrhiza sampled, DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega, Charbonnieres, France). The CTAB extraction buffer was removed, and the ectomycorrhizas were rinsed with sterile water. Two hundred microlitres of Nuclei Lysis Solution were added. Samples were ground into slurry using a micro-homogeniser with sterilised tips and then incubated for 15 min at 65 ºC. Subsequently, 67 µl of protein precipitation solution was added and vortexed. The mixture was centrifuged at 14,000 rpm for 10 min, and the supernatant containing DNA was moved to a fresh tube. The DNA was precipitated by isopropanol and resuspended overnight at 4 ºC in nuclease free water.

Following extraction, the internal transcribed spacer regions I and II and the nuclear 5.8S rRNA gene were amplified using the primer sets ITS-1F/ITS-4B (Gardes and Bruns, 1993) or ITS-1F/ITS-4 (White et al., 1990) depending on amplification success. The PCR mixture for one sample was composed of 15.8 µl of nuclease free water, 5 µl of 10X Buffer, 0.25 µl of each primer (5 µM), 5 µl of deoxynucleotide triphosphate (5 mM) and 0.2 µl of GoTaq. Thermal cycling conditions were as follows: initial denaturalisation at 95ºC for 1 min; 35 cycles at 95ºC for 1 min, 55ºC for 1 min, 72ºC for 1 min; and a final elongation at 72ºC for 10 min. PCR products were assessed by electrophoresis (30 min at 100 W on 1% agarose gels) before sequencing. Gels were stained with ethidium bromide and photographed under ultraviolet light. Unsuccessfully amplified samples were subjected to multiple attempts of PCR at various concentrations of template. The sequencing of the final amplification products was done by MilleGen (Labège, France). Nucleotide sequences were manually edited with the freeware program Chromas Lite 2.01 (http://www.technelysium.com.au/chromas.html).

Ectomycorrhizal identification

Ectomycorrhizal "operational taxonomic units" (OTUs) (Blaxter et al., 2005) were determined as follows: First, edited sequences were examined by BLAST searches against GenBank (http://www.ncbi.nlm.nih.gov/) and the UNITE database (http://unite.zbi.ee/; (Kõljalg et al., 2005)).

Few sequences shared 97% or greater similarity with any of the species deposited in GenBank or UNITE. This cut off level was established based on studies using the ITS region for ECM species identification that have shown that error rates generated by PCR, sequencing and interspecific variability are generally ≤ 3% (Nilsson et al., 2006). Due to the limited number of species identified by BLAST search the samples were grouped by the closest matching genus. For each genus group, sequences were aligned and analysed with MEGA4 (http://www.megasoftware.net/), and the p-distance (% of different nucleotides) was calculated. This measure, although simple, is adequate when studying closely related distances (Nei and Kumar, 2000). Sequences with ≤ 3% of a difference in p-distance were ascribed to a single OTU. The complete name for a species was only given to those OTUs that match a GenBank species sequence by ≥ 97%.

In order to test the grouping and identification of OTUs, phylogenetic trees were generated that included all of the sequences from the same genus and some identified sequences from GenBank as external taxonomic benchmarks. Two types of methods were used for the phylogenetic analysis: 1) the neighbourhood joining method with Kimura-2 distances and confidence assessed by bootstrapping with 500 replicates, and 2) the maximum parsimony method with a heuristic search, tree bisection-reconnection swapping, 100 replicates, gaps treated as 5th character and equal weight of characters methods (Bruns et al., 1998; Nei and Kumar, 2000). These analyses allowed us to test whether the groups identified based on p-distances were right and whether the correspondence of OTUs to identified GenBank species was correct. A last tree in which all of the sequences were analysed together confirmed the correct ascription of the OTUs to families and genera.

Data analysis

Mycorrhizal OTUs were categorised according to their taxonomy (division, order, family and genus) following the Index Fungorum ("www.indexfungorum.org" retrieved 22/06/2008), and fruiting habit, namely, epigeous (aboveground fruiting), hypogeous (belowground fruiting), resupinate (crust-like) and asexual. The abundance for each of these groups was the total number of mycorrhizal tips belonging to each class, while the relative abundance was calculated as the abundance of the group divided by the total number of identified symbionts. The independence in the frequency of categorical variables between oak species-site groups was assessed using the Yates corrected χ^2 test, and the differences in the abundances of categories were compared using the Mann-Whitney U test (as a non parametric alternative for t-test). Occurrence was counted as the number of trees in which fungal OTUs were found.

Species (more precisely OTU) richness (S) was measured as the number of species in a defined sampling unit. Rarefaction analysis was used to estimate the number of species expected in each sample if all samples were of a standard size, thus overcoming the effect of unequal numbers of identified mycorrhizas. The dominance of the community was assessed using Simpson´s diversity index (1-D). Fisher's alpha (α) was also used to measure diversity because of its good discriminant ability and the fact that it is hardly influenced by sample size (Magurran, 1988). All indexes were calculated using Biodiversity Pro V.2 (http://www.sams.ac.uk/research/software).

The taxonomic distinctness index (Δ*), as defined by Warwick and Clarke (1995), was used to estimate the phylogenetic relatedness of the ECM species. It was calculated, based on the topology of a taxonomic tree, as the average path length between any two randomly chosen individuals, conditional on them being from different species. The phylogenetic structure of the ECM communities was statistically assessed using UNIFRAC (Lozupone et al., 2006). A phylogenetic tree was constructed using the neighbour-joining method and both the UniFrac distance metric (Lozupone and Knight, 2005), which is calculated based on differences in the tree branch length, and the P test (Martin, 2002), which estimates similarity between communities based on tree topology, were used to detect differences in the ECM community composition between host species and forest sites.

The similarity of ectomycorrhizal fungal communities between oak trees and between sites was assessed with the Sorensen index. In order to take the distribution of species into account, similarity was also estimated based on abundance with the percentage similarity coefficient according to Jongman et al. (1995).

Two-way factorial ANOVAs using site and host species as categorical factors were performed for each of the measured environmental variables to ascertain if the overall environmental conditions differed significantly under the studied oak species and in both forest sites. When necessary, transformations (logarithmic, square root) were performed to satisfy the necessary assumptions (normality, homoscedasticity). The relationship between the ECM community composition and environmental conditions was analysed by a direct gradient analysis method, the Canonical Correspondence Analysis (CCA), using the Canoco for Windows 4.5 package. This unimodal ordination method was preferred over a linear one (redundancy analysis) since the unconstrained ordination (detrended correspondence analysis, DCA) of the species distribution data showed that they were largely heterogeneous, defining a long main gradient (ter Braak and Smilauer, 2002). Some rare species (singletons) distorted the analysis, producing deviant samples (outliers); thus, we performed the CCA analysis without singletons. Nevertheless, we compared the results with those derived from the analysis done with the complete set of ECM species. Species data were log (x+1) transformed to reduce skewness.

In order to investigate the overall patterns of covariation between the measured variables a d-sep method of path analysis (Shipley, 2000) was used to test for alternative causal models linking the tree (leaf, leaf fall, litter, host identity), soil (topsoil and subsoil) and the ECM fungal community. Each alternative model entails a series of causal assumptions related to the implications of the model for the variances and covariances of the variables that can be tested against the obtained empirical data. A model fits the data when these assumptions can not be falsified (i.e. $p > 0.05$).As a surrogate of the overall ECM community composition we used the main independent gradient of variation extracted with an unconstrained correspondence analysis (CA) on the ECM species data. The CA analysis guaranteed no influence of the environmental variables on gradient selection. Because most variables were not normally distributed we used Spearman correlations in the analysis, for which data was converted to rank.

Since different sets of repeated tests were performed throughout the study a type I error inflation was expected (see García, 2004). To cope with this increased frequency of spurious results, while minimising power losses associated to Bonferroni-related procedures, we controlled the expected proportion of 'false positives' using a FDR (False Discovery Rate) controlling procedure as recommended by García (2003).

RESULTS

ECM community in a Mediterranean forest

A total of 69 different species (OTUs) were identified from the root tips sampled, belonging to 24 genera and 18 families. A detailed list of the identified OTUs is presented in the Supporting Information Table S1. Basidiomycetes dominated the fungal communities on roots of both oak trees (>90% of all identified mycorrhizas) and were present in virtually all sampled trees (23 out of 24). Ascomycetes were scarce, accounting for only 10 mycorrhizas (5% of total abundance), but they were relatively widespread, occurring in 10 of the 24 trees sampled and in both oak species and forest stands. Both fungal groups were similarly distributed among the two oak species and sites ($p > 0.85$; based on Yates corrected χ^2test).

Thelephoraceae was the most diverse family (comprising 28% of the species), followed by Russulaceae (22%) and Cortinariaceae (17%). Eighteen of the 24 identified genera were comprised of only two species. Tomentella and Russula were the most widespread and abundant taxa. The most frequent OTUs were *Lactarius chrysorrheus* (found on seven trees) and *Cenococcum geophilum* (on six trees), which attained 6% and 3% of the total abundance, respectively. Two-thirds of the identified ECM were singletons, occurring only in one sample.

Table 1. Diversity measures of the ECM fungal communities for the combinations of oak host species (*Q. suber* and *Q. canariensis*) and forest sites. Cw: *Q. canariensis*-Woodland, Cf: *Q. canariensis*-Forest, Sw: *Q. suber*-Woodland, and Sf: *Q. suber*-Forest.

	Woodland		Forest		All trees
Diversity measure	*Q. canariensis*	*Q. suber*	*Q. canariensis*	*Q. suber*	
Species richness (S)	22	25	23	23	69
Species density (average spp./ per tree)	4.0	4.8	4.5	6.3	4.9
Simpson´s diversity index (1-D)	0.954	0.954	0.873	0.935	0.965
Rarefaction species richness (n=38)	22.00	21.53	17.35	17.45	
Fisher´s alpha (α)	21.8	21.0	14.3	11.9	35.4
Taxonomic distinctness (Δ)	2.97	3.45	3.28	3.25	3.44
Singleton species	20	19	21	15	46
Similarity index					
between sites:					
Cw-Cf / Sw-Sf	0.13	0.33			
between species:					
Cw-Sw / Cf-Sf	0.13		0.39		

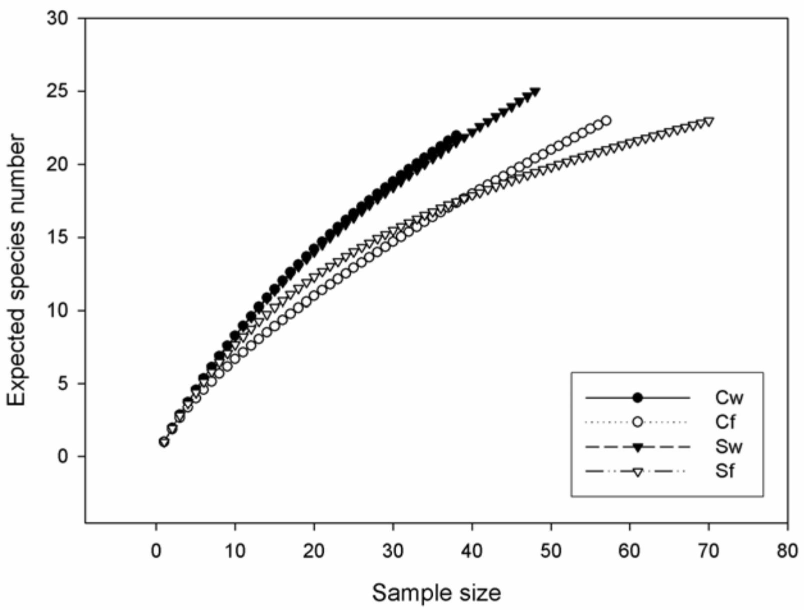

Figure 1. Rarefaction estimation of ECM fungal species richness on roots of *Quercus suber* and *Q. canariensis*. Cw: *Q. canariensis*-Woodland; Cf: *Q. canariensis*-Forest; Sw: *Q. suber*-Woodland; Sf: *Q. suber*-Forest. Sample size is number of ectomycorrhizas.

ECM fungal species with epigeous fruiting bodies were the most diverse (55% of the species) and abundant (60% of identified mycorrhizas) followed by resupinate fungi that attained 27 species and 33% of the total abundance. Hypogeous fungi were scant (3%), and they were rarely found (in 5 oak trees). The frequencies of fruiting habits varied between sites and oak species (Yates corrected χ 2, $p<0.03$).

The total number of ECM species associated with oak trees was similar for the two Quercus species in both study sites (Table 1). Rarefaction analysis indicated a lower diversity of ECM species in the Forest than in the Woodland site, irrespective of the host species (Fig. 1). Other indices, such as the Simpson's diversity index or Fisher's alpha, confirmed this tendency (Table 1). Equal numbers of families (14) and genera (18) were found on roots of both oak species; however, the taxonomic distinctness values were lower for the fungal species colonising the roots of the Algerian oak (*Q. canariensis*) in the Woodland. These results were in agreement with the UniFrac analysis, which suggested that there were significant community shifts between environments overall (UniFrac metric $p<0.026$). Lineage specific analysis revealed that these differences were primarily because of the tomentelloids and russuloids which differed significantly ($p<0.000$) between the four oak-site environments. The ECM community on the roots of *Q. canariensis* in the Woodland site (Cw) was dominated by Tomentella (53% of identified mycorrhizas), followed by Cortinarius (15%). In contrast, Russula was the most abundant taxon (≈35% of the symbionts) on the other three species-site combinations (Cf, Sw and Sf). The community on *Q. suber* in the Forest site (Sf) was dominated by three russuloids, Russula (30%, already mentioned), Lactarius (14%) and Macowanites (10%) (Fig. 2).

Among the 69 identified fungal species, only 13 were found on both Quercus species, e.g., *Cenococcum geophilum*, *Otidea* sp1. and *Byssocorticium atrovirens*, while 29 were recorded exclusively on *Q. canariensis* roots, and 27 species occurred only on *Q. suber*. The limited number of common fungal species between trees resulted in low Sorensen similarity values that ranged from 0.13 to 0.39 (Table 1). Communities from Sf and Cf roots were 35% of similar, and both had about a 17% similarity with Sw. The most dissimilar ECM community was that on the roots of Cw (≈6%), which only shared 4 species with the other groups.

Environmental heterogeneity: oak species and site factors

There were significant differences between forest sites and oak host species for some of the measured environmental variables (Table 2). Soil Ca and pH exhibited highly significant ($p< 0.005$) additive effects for both site and oak species. It is remarkable that litter Ca was the only factor showing both high significant additive and multiplicative (site x oak) effects.

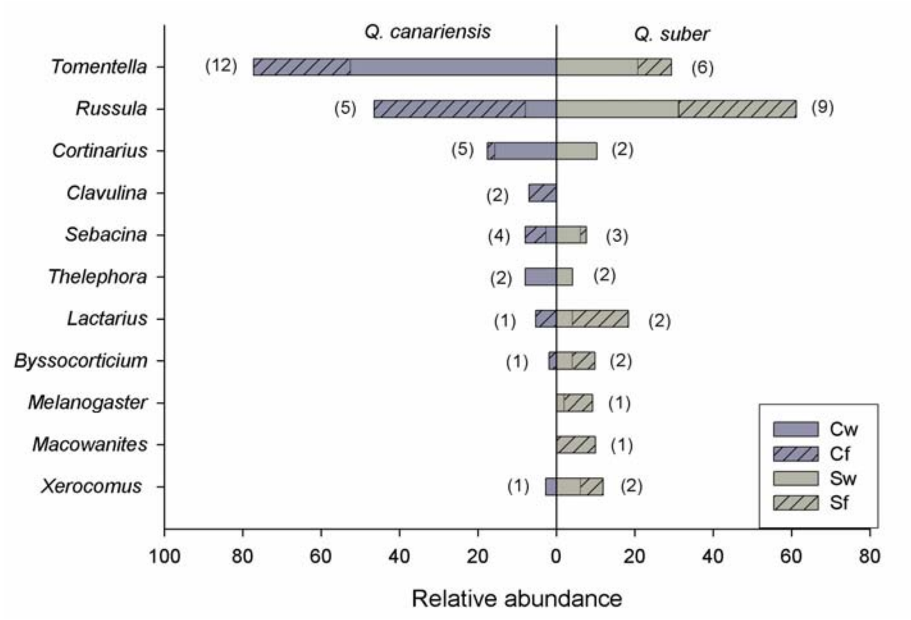

Figure 2. Relative abundance of the most abundant ectomycorrhizal genera on Algerian oak (*Q. canariensis* (C), in the left panel), and cork oak (*Q. suber* (S), in the right panel). The results are grouped by oak host species and forest site (f: Forest, w: Woodland). The number of species per genus is indicated between brackets for each Quercus species.

Litter and topsoil sampled under the canopy of the winter-deciduous species (*Q. canariensis*) were significantly richer in Ca, and the soils were less acidic than those sampled under the evergreen oaks (Table 2). These results were consistent with the differences found in the amount and composition of the leaf fall collected during a whole year under both species (Table 2, third panel), in which Ca was the only element significantly enriched (51% higher) in the leaf fall from the winter-deciduous species. The level of this leaf fall enrichment essentially depended on the oak species because the effects of site and site x oak were not significant.

Regarding the relationships among environmental variables, some very high ($r \geq 0.9$) correlations between litter Ca, soil pH and soil Ca contents were detected, as well as high correlations ($r = 0.8$-0.9) between other several soil and litter variables (Table 3).

Table 2. The mean, standard error and p-values resulting from two-way ANOVA analyses for environmental and complementary variables measured in the topsoil, litter (L) and leaf fall (F) samples collected under the canopy of the two oak host species in the two forest sites. Complementary information on green leaves and subsoil is provided in the lowest panel. The p-values that remained significant after applying a tablewise FDR correction are in bold, and those that are additionally significant on a per-test basis ($p < 0.05$) are in italics.

		Quercus canariensis		*Quercus suber*		Effects significance (p)		
Variable	Unit	Forest	Woodland	Forest	Woodland	Site	Oak	S x O
Litter								
L-Mass	(kg.m^{-2})	1.70 (0.13)	0.86 (0.10)	1.82 (0.20)	1.04 (0.23)	0.00	0.40	0.86
L-N	(mg.g^{-1})	10.5 (0.6)	9.9 (0.6)	11.6 (0.5)	9.5 (0.5)	*0.03*	0.54	0.19
L-Ca	(mg.g^{-1})	13.1 (1.0)	25.2 (0.8)	9.1 (0.6)	12.0 (0.7)	**0.00**	**0.00**	**0.00**
L-K	(mg.g^{-1})	1.38 (0.03)	1.71 (0.06)	1.16 (0.07)	1.38 (0.10)	**0.00**	**0.00**	0.43
L-Mg	(mg.g^{-1})	1.59 (0.12)	2.07 (0.15)	1.28 (0.04)	1.44 (0.03)	**0.01**	**0.00**	0.13
L-P	(mg.g^{-1})	0.45 (0.03)	0.58 (0.03)	0.45 (0.03)	0.47 (0.03)	*0.03*	0.11	0.12
L-S	(mg.g^{-1})	0.12 (0.00)	0.13 (0.00)	0.12 (0.00)	0.11 (0.00)	0.36	**0.02**	0.13
Topsoil								
pH		4.97 (0.07)	5.85 (0.07)	4.54 (0.08)	5.18 (0.10)	**0.00**	**0.00**	0.15
C	(mg.g^{-1})	51.1 (12.1)	60.4 (7.6)	38.2 (5.0)	45.4 (2.6)	0.12	0.10	0.91
N	(mg.g^{-1})	2.41 (0.31)	2.88 (0.22)	2.04 (0.26)	2.01 (0.16)	0.37	*0.02*	0.31
Ca	(mg.g^{-1})	1.30 (0.46)	3.27 (0.36)	0.48 (0.14)	1.75 (0.37)	**0.00**	**0.00**	0.97
K	(mg.g^{-1})	1.12 (0.08)	3.57 (0.42)	1.53 (0.22)	2.85 (0.40)	**0.00**	0.50	*0.02*
Mg	(mg.g^{-1})	1.01 (0.06)	3.36 (0.35)	1.19 (0.18)	2.97 (0.36)	**0.00**	0.45	0.19
P	(mg.g^{-1})	0.21 (0.02)	0.30 (0.02)	0.21(0.02)	0.25 (0.02)	**0.00**	0.15	0.13
P-Bray	(mg.kg^{-1})	5.45 (0.56)	3.16 (0.93)	2.59 (0.38)	2.77 (0.58)	0.10	*0.04*	0.10
S	(mg.kg^{-1})	0.24 (0.01)	0.24 (0.02)	0.23 (0.02)	0.19 (0.01)	0.64	0.07	0.39
Leaf fall								
F-Mass	(kg.m^{-2}.yr^{-1})	0.32 (0.02)	0.31 (0.03)	0.31 (0.01)	0.26 (0.01)	**0.00**	0.74	**0.01**
F-N	(mg.g^{-1})	10.5 (0.9)	8.8 (0.2)	10.1 (0.8)	8.4 (0.1)	**0.01**	0.52	0.99
F-Ca	(mg.g^{-1})	12.8 (0.9)	14.4 (0.2)	8.8 (1.0)	9.2 (1.0)	0.20	**0.00**	0.49
F-K	(mg.g^{-1})	2.81 (0.24)	1.69 (0.17)	3.11 (0.34)	1.86 (0.16)	**0.00**	0.31	0.76
F-Mg	(mg.g^{-1})	1.95 (0.20)	1.50 (0.09)	1.50 (0.07)	1.27 (0.06)	**0.01**	**0.01**	0.39
F-P	(mg.g^{-1})	0.63 (0.08)	0.47 (0.02)	0.52 (0.03)	0.35 (0.02)	**0.00**	*0.02*	0.40
F-S	(mg.g^{-1})	1.69 (0.11)	1.11 (0.01)	1.15 (0.05)	0.95 (0.03)	**0.00**	**0.02**	0.63
Complementary data								
Ca in subsoil	(mg.g^{-1})	0.23 (0.07)	2.40 (0.83)	0.27 (0.06)	1.06(0.34)	**0.00**	0.41	0.08
Ca-Top/Ca-sub		5.78(0.56)	2.64(0.78)	1.73(0.30)	2.05(0.38)	0.05	0.05	**0.00**
Ca in leaves	(mg.g^{-1})	8.4 (0.5)	11.0 (1.5)	3.3 (0.3)	4.2 (0.2)	*0.03*	**0.00**	0.87

Table 3. High correlations (● = 0.8-0.9, ●●> 0.9) found between soil and litter (L-) variables. All marked correlations were positive.

	L-Ca	L-K	L-Mg	pH	C	N	Ca	K	Mg
L-K	●								
L-Mg	●	●							
pH	●●								
C									
N					●●				
Ca	●●		●	●●					
K									
Mg				●			●	●●	
P						●		●	●

ECM community-environment relationships

The distribution of the ECM species was explained by significant conditional effects of the forest site ($p< 0.023$), the host oak species ($p< 0.038$) and the site x host interaction ($p< 0.036$), as shown by the factorial CCA. The overall CCA factorial model explained 19.6% of the total ECM species variance and was highly significant ($p< 0.0023$).

When the measured topsoil variables were used as predictors of the ECM species distribution alone, only two variables showed highly significant ($p< 0.01$) marginal effects: soil pH ($p< 0.0007$) and soil Ca content ($p < 0.0041$). Because of the very high correlation between these two variables (Table 3) only the pH had a significant conditional effect and thus was retained as the best predictor. This soil variable alone was able to explain 8.2% of the overall ECM species variance.

On the other hand, when the litter variables were used in the CCA analysis only litter Ca showed a highly significant ($p< 0.01$) conditional effect as a predictor of the ECM community structure. Furthermore, when both the soil and litter variables were included in the analysis, the model based on litter Ca content alone ($p< 0.0002$, 8.4% overall variance explained) outperformed all other CCA models based on any combination of the remaining measured soil and litter variables, including soil pH. The close relationship between litter Ca and soil pH (Table 3) and the better predictive performance of the litter Ca explained why only this variable was retained as a meaningful predictor of the ECM community structure.

In order to ascertain whether the hypothesized interactions between host species, soil and litter conditions and ECM fungal communities had a significant empirical support we used d-sep analysis to test different alternative causal models which underlying hypothesis were: 1) Main variations in the ECM communities may be explained by soil changes derived only from differences in intrinsic soil properties (subsoil), 2) Only host identity affects the ECM community composition by means of a direct genetic specificity, 3) There is a species indirect effect via differential leaf, leaf fall and litter quality that explains the main ECM variation patterns and 4) The combination of direct and indirect species effects and the primary soil properties drives the ECM community composition. All path diagrams corresponding to the proposed models are shown in Fig. 3. Calcium concentration in leaves, leaf fall, litter, topsoil and subsoil were used as surrogate variables in the models.

Table 4 includes the results of the analysis of the tested causal models. Only models explicitly considering the indirect effects of host species on ECM communities, via changes in litter (or litter and topsoil) composition matched the overall available empirical evidence (Fig. 3). Nevertheless the model based on simultaneous direct and indirect host influence was clearly more consistent with

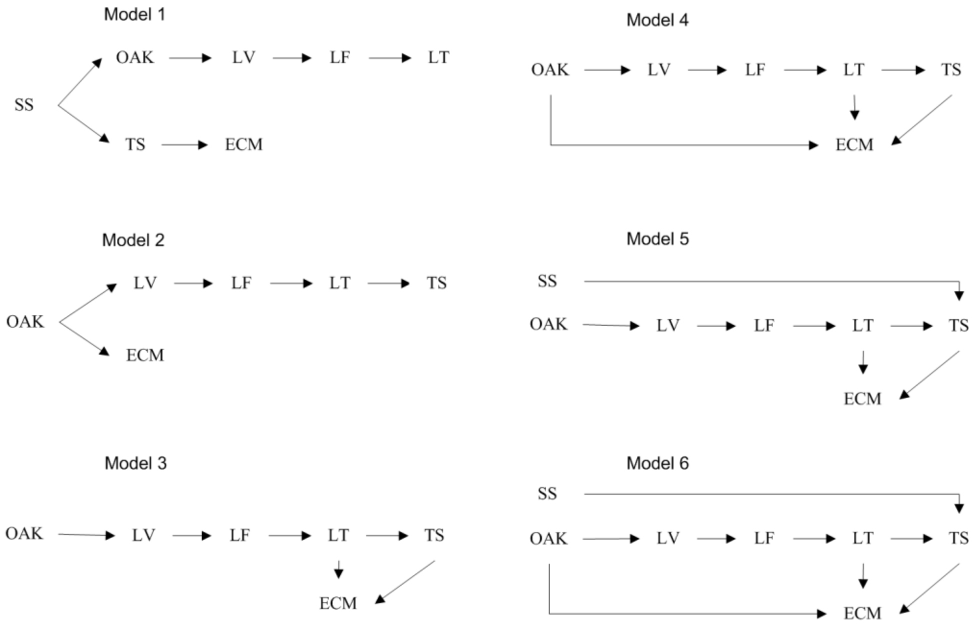

Figure 3. Alternative causal models used to fit the dataset of host species (OAK), the environmental variables (calcium content of the subsoil (SS), green leaves (LV), leaf fall (LF), litter (LT) and pH of the topsoil (TS)) and ECM community composition (expressed as the 1st CA axis) to explain the patterns of variation. Model 1: Direct effect of soil intrinsic properties; Model 2: Direct effect of host genetic specificity; Model 3: Indirect effect of host species via leaf fall and litter quality; Model 4: Direct and indirect effects of host species; Model 5: Direct effect of soil intrinsic properties and indirect effect of host species; Model 6: Direct effect of soil intrinsic properties and direct and indirect effects of host species.

Table 4. Shipley's d-sep test results of different causal models which could potentially explain the observed covariation patterns among host species (*Q. canariensis*,*Q. suber*), Ca content in different forest compartments (green leaves, leaf fall, litter fall, subsoil and topsoil) and ECM fungal community structure (summarised by the main axis extracted by correspondence analysis from the ECM data).

Model	Chi-sq	d.f.	p	Tested effect
1	92.05	30	0.0000	Direct effect of soil intrinsic properties
2	32.80	20	0.0354	Direct effect of host genetic specificity
3	28.30	18	**0.0577**	Indirect effect of host species *via*leaf fall and litter quality
4	20.16	16	**0.2131**	Direct and indirect effects of host species
5	56.68	28	0.0011	Direct effect of soil intrinsic properties and indirect effect of host species
6	43.50	26	0.0171	Direct effect of soil intrinsic properties and direct and indirect effects of host species

the empirical evidence ($p < 0.21$) than the one supported only by indirect effects ($p < 0.06$). All the models considering the independent influence of primary soil properties on the ECM communities were rejected.

Environmental conditions and diversity of the ECM communities

The overall mycorrhizal species richness increased significantly with soil acidity ($r = 0.58$, $p < 0.003$). Negative relationships were found between the number of ECM genera and families and the litter Ca concentration ($r = -0.43$, $p < 0.034$) indicating a significantly lower taxonomic diversity with increasing Ca availability and soil pH.

The distribution of taxa according to their fruiting habit was also significantly related to environmental conditions. The diversity and abundance of epigeous populations was negatively correlated with Ca in litter ($r \approx -0.50$, $p < 0.03$) and soil pH ($r \approx -0.57$, $p < 0.03$). The opposite pattern was observed for resupinate species, for which richness rose along the gradient of Ca in litter ($r = 0.44$, $p < 0.03$) and soil pH ($r = 0.47$, $p < 0.02$).

DISCUSSION

ECM fungal communities in a Mediterranean forest

We have found that southern Spain oak forests harbour a relatively high ECM fungal species richness: 69 OTUs belonging to 24 genera and 18 families were identified in the roots of two oak species in two forest sites. These richness values are in accordance with others found in oak forests (ranges from 39 to 79; (Avis et al., 2003; Walker et al., 2005) although lower than the maximum values of 95-140 taxa recorded in some Mediterranean forests (Richard et al., 2004; Morris et al., 2008). The species-area curve obtained here (Fig. 1) indicates that the local ECM fungal biodiversity must be even higher. We found a large number of rare taxa (46 singleton species) that is a common feature in ECM fungal communities (Kõljalg et al., 2000; Avis et al., 2008).

Thelephoraceae (28% of the species), Russulaceae (22%) and Cortinariaceae (17%) were the most diverse and abundant families in the ECM fungal communities, as has also been found in other forests (Gardes and Bruns, 1996; Richard et al., 2005; Riviere et al., 2007). In a similar study, Morris et al. (2008) investigated the ECM fungal community on the root of two coexisting oak species in a California woodland, and found a large abundance of Ascomycota (40%) and hypogeous fruiting fungi (~25%). In contrast, a low abundance of these groups was recorded in Mediterranean Quercus forests of south Spain (this study) and Corsica (Richard et al. 2005). These strong differences between ECM fungal communities on similar oak species and under mediterranean-type climate, but in distant biogeographical regions (California versus Mediterranean Basin), are worth to be studied in more detail. The Ascomycota species *Cenococcum geophilum*, which is a known associate of Quercus roots (Dickie et al., 2004; Walker et al., 2005), only represented 3% of the total abundance in the forests studied. The relative humidity and high precipitation level in the study sites may negatively impact the colonisation and spread of this drought tolerant fungus (Pigott, 1982). In addition, the small size of *C. geophillum* ECM tips may cause the unsuccessful amplification of their DNA and thus, result in their under-representation (Smith et al., 2007).

Indirect effects of host species on ECM fungal communities

One of the main findings of this study is that the oak host species may indirectly affect ECM fungal communities through their leaf fall and litter quality, as is suggested by the tested causal models. In general, the species-specific effects of trees on biogeochemical cycles and ecosystem processes have long been recognized (Zinke, 1962; Hobbie, 1992; Finzi et al., 1998). In the two forests studied the calcium content in litter and soil, together with soil pH, emerged as the most influential variables for mediating indirect host-species effects on ECM fungal communities since: i) they were the only significant variables explaining the ECM fungal community patterns even when other potentially influential factors (soil C, N, and P) were also studied; ii) they exhibited a highly significant oak species effect irrespective of the homogeneous subsoil conditions; iii) significant differences in the leaves and leaf fall Ca -that were exclusively related to the oak species- were observed before the leaf fall interacts with the soil surface; and iv) they are conceptually related, since increased Ca inputs can raise exchangeable base saturation and mitigate soil acidity. Accordingly, the winter-deciduous oak (*Q. canariensis*) was able to pump from the soil significantly more Ca than the coexisting evergreen species (*Q. suber*) leading to a top-down cascading effect by which the relative Ca enrichment in the materials collected under the winter-deciduous species in comparison to the evergreen *Q. suber* increased from leaf fall (51% higher) to litter (81% higher) to topsoil (251% higher). We suggest that the differences in leaf fall Ca-levels, and their yearly accumulation and incorporation into the litter, yield increasingly distinct litter and topsoil, thus producing selective environmental conditions that shape the ECM fungal community. The observed shift in the dominant taxa from russuloids to tomentelloids was the most outstanding change associated with the oak-induced variation in the environmental conditions and it was observed in the two forest sites despite their contrasting environmental conditions.

Leaf fall calcium content is a tree species-specific trait that depends on their uptake, allocation and retention capacity; thus, even species growing under similar subsoil nutrient supplies, as it occurred in the studied forests, in time, distinctly modify their soil abiotic environments (Reich, 2005). Leaf fall Ca determines litter Ca, which has a strong relationship with forest floor biogeochemical dynamics such as soil acidity, base saturation, C and N contents, decomposition rates and soil heterotrophic community composition (Reich, 2005; Hobbie et al., 2006). Since soil pH was closely correlated to calcium levels in soil and litter, and since it was also a significant predictor for ECM fungal species distribution, calcium-induced changes in soil acidity seem to play a main role in determining the observed differences in the fungal communities between environments. The relationship between shifts in ECM fungal species communities and soil pH has been previously described: Soil acidity seems to have a species-specific effect on the production of fruiting bodies (Agerer et al., 1998). In vitro experiments have shown that substrate pH affects the growth capacity (Hung and Trappe, 1983), mycelial density (Rosling et al., 2004), colonization potential (Erland and Soderstrom, 1990) and enzymatic capabilities (Courty et al., 2005) of mycorrhizal species. Changes in the performance of species under different pH levels affect their competitive abilities (McAfee and Fortin, 1987), altering their relative abundance in the community. However these changes were treated as an abiotic host-independent influence and were not related to host indirect effects through leaf fall or litter quality.

ECM fungal diversity and soil conditions

No significant changes in ECM fungal species richness were observed between oak species, and forest sites. However, the taxonomic diversity (number of genera and families) and the phylogenetic structure of the community significantly shifted. Other studies have also found changes in species dominance from epigeous to resupinate (Peter et al., 2001), and from Basidiomycetes to Ascomycetes (Gehring et al., 1998) related to variations in soil conditions (e.g. increase in nutrient availability). Differences in the abundance and diversity of epigeous species between the roots of evergreen and deciduous coexisting oaks were also recently observed by Morris et al. (2008).

The lower ECM fungal taxonomic diversity recorded on the roots of *Q. canariensis* in the Woodland site was mainly due to both the higher abundance of resupinate tomentelloid species and the disappearance of epigeous taxa. It is possible that the prevailing environmental conditions, i.e. higher litter, and soil nutrient content and pH, may be limiting for certain fungal species (e.g. Russula) and thus represent an environmental filter yielding a phylogenetic clustering of closely related "tolerant" species such as the tomentelloids.

Environmental selection for biological traits conferring tolerance and selection for competitive interactions that promote functional diversification are two of the main processes that govern the phylogenetic structures of animal, plant and bacterial communities (Fox and Brown, 1993; Weiher and Keddy, 1995; Horner-Devine and Bohannan, 2006). The relationships that we have found between ECM fungal taxonomic diversity and soil conditions in these mixed oak forests suggest that these complementary ecological processes might also drive the community structure of mycorrhizal fungi.

CONCLUSIONS

Our work suggests that leaf fall-mediated indirect host-effects may play a critical role in determining the ECM fungal community assemblage and taxonomic diversity. These effects could have been interpreted either as pure environmental effects (host-independent), if the relationships between soil properties and the host source materials (leaf fall, litter) had not been simultaneously investigated, or as a generic mixed host-site effect, if only a factorial multivariate analysis had been performed. To our knowledge this is the first attempt to investigate the indirect effect of host species on ECM fungal assemblages. Since this interaction had been previously unnoticed and seems to be superimposed to direct host effects, further work is needed to unveil its relative importance as a driving factor of the ECM fungal communities.

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APPENDIX

Table S1. Absolute abundance and occurrence of fungal OTUs recovered from oak roots, grouped by families. The outputs of the GenBank search are: closest match, closest match accession number, and nucleotide similarity.

OTU	Abundance	Occurrence	Closest GenBank match	% Similarity	Accession Nº
Atheliaceae					
Byssocorticium atrovirens	6	5	*Byssocorticium atrovirens* (AJ889936.1)	697/699 (99%)	FJ946963
Byssocorticium sp.1	1	1	Corticiaceae (DQ990867.1)	559/589 (94%)	FJ946949
Bolbitiaceae					
Hebeloma ammophilum	2	2	*Hebeloma* (EF411103.1)	727/731 (99%)	FJ946936
Boletaceae					
Boletus sp	1	1	*Xerocomus chrysenteron* (AJ419223.1; XCH419223)	267/320 (83%)	FJ946971
Xerocomus chrysenteron	3	1	*Xerocomus chrysenteron* (AJ419223.1; XCH419223)	645/652 (98%)	FJ946978
Xerocomus subtomentosus	5	4	*Xerocomus subtomentosus* (DQ066366.1)	694/746 (93%)	FJ946972

Clavulinaceae					
Clavulina sp. 1	1	1	Clavulinaceae (AJ534708.1; CSP534708)	700/710 (98%)	FJ946959
Clavulina sp. 2	3	1	*Clavulina cristata* (AY292292.1)	485/638 (76%)	FJ946967
Cortinariaceae					
Alnicola sp.	1	1	*Alnicola cf. scolecina* (AY303583.1)	515/597 (86%)	FJ946921
Cortinariaceae	1	1	*Cuphocybe sp.* (AY669629.1)	731/774 (94%)	FJ946923
Cortinarius cephalixus	1	1	*Cortinarius cephalixus* (AY174786.1)	721/721(100%)	FJ946968
Cortinarius dionysae	4	1	*Cortinarius dionysae*(AY174813.1)	756/770 (98%)	FJ946938
Cortinarius sp.1	1	1	*Cortinarius tortuosus* (AY669669.1)	626/669 (93%)	FJ946948
Cortinarius sp.2	1	1	*Cortinarius parvannulatus* (AY669664.1)	541/660 (81%)	FJ946934
Cortinarius sp.3	1	1	*Cortinarius* (EF218748.1)	622/707 (87%)	FJ946920
Cortinarius umbrinolens	4	1	Uncultured fungus (EU292639.1)	533/551 (96%)	FJ946918
Cenococcum[1]					
Cenococcum geophilum	6	6	*Cortinarius* (DQ481865.1)	459/489 (93%)	FJ946957
Helotiales					
Helotiales sp.	1	1	Pezizomycotina (DQ273333.1)	485/508 (95%)	FJ946951
Inocybaceae					
Inocybe sp.1	1	1	*Cortinarius* (AY825511.1)	632/646 (97%)	FJ946937
Inocybe sp.2	1	1	*Inocybe maculate* (AJ534933.1; FA534933)	609/728 (83%)	FJ946943
Inocybe sp.3	1	1	*Inocybe* (EF417815.1)	665/775 (85%)	FJ946983
Melanogastraceae					
Melanogaster variegates	6	4	*Melanogaster macrosporus* (AJ555528.1; MMA555528)	460/474 (97%)	FJ946956
Peniophoraceae					
Peniophora sp	1	1	*Peniophora pini* (EU118651.1)	652/716 (91%)	FJ946985
Pluteaceae					
Amanita citrina	4	3	*Amanita* (DQ990869.1)	490/511 (95%)	FJ946974
Amanita pantherina	1	1	*Amanita gemmata* (AF335440.1; AF335440)	723/764 (94%)	FJ946976
Pyronemataceae					
Humaria sp.	1	1	Uncultured ectomycorrhiza (EU403109.1)	630/638 (98%)	FJ946919
Otidea sp.	3	2	*Otidea smithii* (AF072064.1; AF072064)	341/373 (91%)	FJ946964
Russulaceae					
Lactarius chrysorrheus	13	7	*Lactarius luculentus* (EU486450.1)	779/821 (94%)	FJ946981
Lactarius sp.	2	1	*Lactarius eucalypti* (EU019923.1)	513/522 (98%)	FJ946984
Macowanites ammophilus	7	2	*Russula amoenolens* (DQ822824.1)	674/695 (96%)	FJ946980
Russula cf. emetica	11	5	*Russula cf. emetica* (AY228360.1)	735/756 (97%)	FJ946940
Russula densifolia	1	1	*Russula densifolia* (AY606961.1)	628/631 (99%)	FJ946973
Russula persicina	2	1	*Russula persicina* (DQ422019.1)	616/630 (97%)	FJ946942
Russula risigallina	1	1	*Russula risigallina* (DQ422022.1)	584/597 (97%)	FJ946975
Russula sp.1	29	3	Russulaceae (DQ061882.1)	758/771 (98%)	FJ946961
Russula sp.2	3	1	*Russula* (EF218805.1)	711/735 (96%)	FJ946960
Russula sp.3	1	1	*Russula acrifolia* (DQ421998.1)	449/599 (74%)	FJ946962
Russula sp.4	1	1	*Russula* (AJ893219.1)	697/758 (91%)	FJ946944
Russula sp.5	1	1	*Russula* (EF641837.1)	546/631 (86%)	FJ946982
Russula sp.6	5	1	Russulaceae (DQ061902.1)	498/633 (78%)	FJ946947
Russula sp.7	3	3	Uncultured fungus (DQ054555.1)	740/775 (95%)	FJ946932

Russula vesca	3	3	Russulaceae (AY534202.1)	725/756 (95%)	FJ946977
Sebacinaceae					
Sebacina epigaea	1	1	Sebacinales (EF417818.1)	554/558 (99%)	FJ946950
Sebacina helvelloides	1	1	Sebacinales (EF030882.1)	566/577 (98%)	FJ946922
Sebacina sp.1	2	2	Sebacinaceae (AY825519.1)	562/566 (99%),	FJ946958
Sebacina sp.2	3	3	Sebacinales (EF417803.1)	644/706 (91%)	FJ946952
Sebacina sp.3	1	1	Sebacinaceae (AF465185.1)	588/611 (96%)	FJ946941
Thelephoraceae					
Thelephora sp.1	2	1	Thelephoraceae (AJ893325.1)	746/753 (99%)	FJ946935
Thelephora sp.2	2	2	Thelephoraceae (EF411075.1)	684/755 (90%)	FJ946927
Thelephora sp.3	1	1	Uncultured ectomycorrhiza (AB218132.1)	454/508 (89%)	FJ946945
Tomentella sp.1	6	2	Thelephoraceae (AJ893313.1)	708/751 (94%)	FJ946926
Tomentella sp.2	4	1	*Tomentella* (EF218835.1)	736/753 (97%)	FJ946917
Tomentella sp.3	2	1	Thelephoraceae (DQ273420.1)	602/619 (97%)	FJ946954
Tomentella sp.4	7	4	Thelephoraceae (AJ893308.1)	751/755 (99%)	FJ946946
Tomentella sp.5	2	1	*Tomentella* (AF430289.1)	735/749 (98%)	FJ946924
Tomentella sp.6	3	2	Thelephoraceae (EF619772.1)	531/554 (95%)	FJ946939
Tomentella sp.7	2	1	Thelephoraceae (EF411074.1)	693/762 (90%)	FJ946931
Tomentella sp.8	2	2	Uncultured fungus (EF040848.1)	606/608 (99%)	FJ946965
Tomentella sp.9	1	1	Thelephoraceae (AJ893343.1)	723/753 (96%)	FJ946929
Tomentella sp.10	11	2	*Tomentella* (U92537.1; TSU92537)	586/626 (93%)	FJ946970
Tomentella sp.11	1	1	Thelephoraceae (EF411072.1)	729/750 (97%)	FJ946979
Tomentella sp.12	1	1	Thelephoraceae (EF411078.1)	507/526 (96%)	FJ946933
Tomentella sp.13	1	1	Thelephoraceae (EF411123.1)	579/742 (78%)	FJ946930
Tomentella sp.14	1	1	Thelephoraceae (AJ893332.1)	736/749 (98%)	FJ946925
Tomentella sp.15	2	2	Thelephoraceae (AJ893307.1)	739/760 (97%)	FJ946928
Tomentella sublilacina	4	1	Thelephorales (DQ195590.1)	731/753 (97%)	FJ946955
Tricholomataceae					
Tricholoma sp	3	2	*Tricholoma* (EU057088.1)	749/789 (94%)	FJ946969
Tuberaceae					
Tuber sp.	1	1	*Tuber borchii* (DQ402505.1)	432/465 (92%)	FJ946966
Unidentified sp					
Unidentified sp (hysterangium)	1	1	Uncultured ectomycorrhiza (AB251842.1)	487/580 (83%)	FJ946953

[1] *Cenococcum* is listed at the genus level because of its unresolved classification

Capítulo 8.

Discusión general

Capítulo 8. **Discusión general**

Los ecosistemas terrestres están formados por dos subsistemas, la parte aérea (*aboveground*) y el suelo o parte subterránea (*belowground*), que dependen necesariamente uno del otro. Toda aproximación que trate de comprender el funcionamiento de dichos ecosistemas requiere la consideración explícita de ambos subsistemas. En esta tesis se aborda el estudio de las relaciones que se establecen entre las plantas (un componente principal del subsistema aéreo) y el suelo con el objetivo de conocer las interacciones más relevantes entre ambos, elucidar los posibles procesos de retroalimentación que existen entre las comunidades de plantas leñosas y el suelo interpretar cómo estos dos componentes gobiernan conjuntamente el funcionamiento del ecosistema.

Para comprender cómo la comunidad de plantas leñosas influye sobre las comunidades y los procesos del suelo es necesario comprender, primero, cómo y porqué las distintas especies de la comunidad forestal difieren en sus efectos sobre el suelo.

RASGOS FOLIARES: VARIABILIDAD Y FUNCIÓN

La comunidad de especies forestales estudiada muestra una elevada variabilidad interespecífica en los rasgos foliares, reflejo de la diversidad funcional de las especies que la componen (Ackerly, 2004). También se observa una amplia variabilidad intraespecífica en los mismos rasgos, resultado de la interacción entre las diferencias genotípicas de los individuos y las condiciones del medio (Garten, 1978; Peñuelas et al., 2008). Estas interacciones determinan la morfología y composición química foliar de los individuos y generan unos gradientes de variación en el ecosistema que, como hemos observado, tienen importantes repercusiones en la comunidad de organismos del suelo y, por tanto, en los procesos que de éstos dependen como la descomposición y mineralización de la materia orgánica (Madritch y Hunter, 2002, 2005). El efecto que los individuos generan en el funcionamiento del ecosistema, fruto de la interacción entre su genotipo y las condiciones ambientales, se ha definido como su fenotipo extendido (*extended phenotype*) (Whitham et al., 2003; Cianciaruso et al., 2009). La influencia de cada genotipo en las comunidades del suelo y los procesos que éstas controlan, es decir, su fenotipo extendido, está sujeto a selección natural y puede ser heredado por subsecuentes generaciones de plantas, aunque las implicaciones de esta heredabilidad son aún poco conocidas (Schweitzer et al., 2008). Los estudios de interacciones ecológicas deben avanzar en la integración de las características genéticas en los procesos de retroalimentación entre las plantas y los organismos del suelo (Bardgett y Wardle, 2010).

La variabilidad interespecífica de los rasgos foliares químicos resultó ser superior a la de los rasgos morfológicos, en particular el contenido en micronutrientes y elementos no esenciales. La distinta variabilidad en los macro y micro elementos no se observó entre los individuos de quercíneas estudiados. Las plantas ejercen un control desigual sobre sus rasgos foliares que se refleja en la distinta variabilidad de estos rasgos. La relativa baja variabilidad observada en la comunidad de la masa por unidad de área (LMA, del inglés *leaf mass per area*), el contenido de carbono (LCC, del inglés *leaf carbon content*) o el contenido del isótopo $\delta^{13}C$ sugiere que estas variables están sujetas a un mayor control biológico, posiblemente debido a que estos caracteres juegan un papel clave en la ecofisiología de las plantas (Seibt et al., 2008; Poorter et al., 2009). En cuanto al contenido de macronutrientes en el tejido foliar se distinguen, a nivel inter e intraespecífico, elementos muy poco variables, como el N, S y P, frente a otros con mayor variabilidad como el Ca, Mg y K. Esta distinción es consistente con la observada en otros ecosistemas forestales y se atribuye a diferencias en sus ciclos biogeoquímicos y a la relevancia de sus funciones fisiológicas (Salisbury y Ross, 1991; Merila y Derome, 2008; Ladanai et al., 2010). Los micronutrientes y minerales no esenciales presentaron una gran variabilidad entre las especies estudiadas, pero no entre los 50 individuos de las dos especies de quercíneas. La absorción de estos elementos está poco controlada por la planta y su acumulación en el tejido foliar, en ocasiones superior a la necesaria, depende de las concentraciones en el suelo y de las diferencias específicas en la capacidad de absorción o retención en las raíces, provocando elevadas diferencias entre especies pero no entre individuos (Madejón et al., 2006; Watanabe et al., 2007; Domínguez et al., 2009).

A pesar de la distinta variabilidad observada en los elementos químicos estudiados, su concentración en el tejido foliar mostró un importante grado de correlación, tanto entre especies como entre individuos, especialmente para los elementos menos variables. La baja variabilidad de elementos altamente correlacionados sugiere que determinados contenidos elementales no son aleatorios sino que responden a una funcionalidad biogeoquímica en el metabolismo celular que se traduce en unas relaciones estequiométricas relativamente fijas (Knecht y Goransson, 2004). Los resultados aquí presentados y otros estudios recientes han demostrado la existencia de este tipo de relaciones estequiométricas en plantas, suelos y masa microbiana que indican que la estequiometría de estos organismos se encuentra constreñida por la función fisiológica (Ågren, 2004; Cleveland y Liptzin, 2007). Recientemente se ha despertado un gran interés por la estequiometría ecológica que investiga cómo el balance químico interno de los organismos puede interaccionar y afectar a los procesos del ecosistema. Especies vegetales con un funcionamiento ecofisiológico parecido exhibirán unas proporciones químicas similares que determinarán el impacto de estas especies en los ciclos biogeoquímicos (Baxter et al., 2008; Sterner y Elser, 2009).

Tanto a nivel de comunidad como a nivel intraespecífico se ha observado un amplio espectro de rasgos funcionales, morfológicos y químicos, y una relación significativa entre ellos. La combinación de rasgos que exhiben las especies es el resultado de su origen filogenético y de las adaptaciones a las condiciones del medio y, por tanto estará relacionada con el comportamiento ecofisiológico de las especies con respecto a los recursos (Lavorel y Garnier, 2002; Diaz et al., 2004). Las especies con una estrategia "adquisitiva" de recursos presentan un conjunto de rasgos funcionales que les permiten una mayor captación de los recursos y un crecimiento más rápido que aquéllas con una estrategia "conservadora" que minimizan la captación, uso y pérdida de recursos a cambio de un crecimiento más lento (Poorter y Garnier, 1999; Wright y Westoby, 2001; Wright et al., 2004). Los rasgos foliares, particularmente los relacionados con la adquisición de recursos, son determinantes de los efectos que las distintas especies vegetales tienen sobre las propiedades del ecosistema, pues modulan la disponibilidad de recursos como la luz, determinan la calidad de la hojarasca y los procesos biogeoquímicos que dependen de ésta y, en último término, influyen en el reclutamiento de las especies y en la dinámica forestal (Chapin III, 2003; Diaz et al., 2004; Cornelissen et al., 2006; Cornwell et al., 2008). Trabajos recientes sugieren que los rasgos funcionales de los sistemas radicales podrían estar relacionados con los rasgos foliares de modo que ambos ejercen una influencia similar en los procesos del ecosistema (Freschet et al., 2010; Liu et al., 2010). Es importante continuar ampliando el conocimiento de las interacciones entre los rasgos funcionales de las especies vegetales y el funcionamiento del ecosistema para logar un mayor conocimiento de las reglas de ensamblaje de la comunidad, conocer qué rasgos son determinantes en los procesos de ecosistema, detectar cambios relevantes en la composición de las comunidades y comprender las repercusiones que estos cambios tienen en el ecosistema.

MECANISMOS DE INTERACCIÓN ENTRE ÁRBOL Y SUELO

Muchos trabajos han mostrado que las especies vegetales difieren en los efectos que generan en las comunidades de organismos y en las propiedades del suelo (Binkley y Valentine, 1991; Finzi et al., 1998a; Augusto et al., 2002). Sin embargo entender cuáles son los mecanismos que subyacen a estos efectos específicos y su implicación en los procesos del ecosistema es esencial para comprender el funcionamiento de los ecosistemas (Bardgett y Wardle, 2010). Para ello nos hemos centrado en el estudio de las interacciones entre las dos especies de quercíneas presentes en la comunidad, alcornoque (*Q. suber*) y quejigo moruno (*Q. canariensis*), y el suelo sobre el que se desarrollan, mediadas por las características de su desfronde. Las diferencias entre los rasgos foliares de estas especies son notables debido a su contrastado hábito foliar (perenne frente a caducifolia), aunque dentro del amplio espectro de la comunidad de plantas leñosas estas especies (congéneres) no parezcan tan distantes. Todas las especies que conforman una comunidad vegetal influyen en las propiedades del ecosistema pero es razonable asumir que la relevancia del efecto será relativa a su contribución total a la biomasa de la comunidad (Grime, 1998). En ocasiones, sin embargo, determinadas especies producen efectos desproporcionados a su masa que afectan de modo substancial a la comunidad (Ehrenfeld et al., 2001; Gómez-Aparicio y Canham, 2008; Rodgers et al., 2008; Fischer et al., 2010).

Los resultados han demostrado que las dos especies de quercíneas estudiadas, a través de sus contrastados rasgos foliares, generan distintos efectos en los ciclos biogeoquímicos del ecosistema, alterando las condiciones bióticas y abióticas del suelo y dando a lugar a potenciales ciclos de retroalimentación (Figura 1). El análisis multivariante conjunto de la composición química de los componentes del ecosistema (hoja viva, desfronde, hojarasca y suelo) reveló una estrecha covariación en los patrones de enriquecimiento de nutrientes, lo que sugiere una transferencia directa de estos elementos entre la planta y el suelo. Ambas especies mostraron una separación significativa en el espacio multivariante definido por la composición química del conjunto de los componentes estudiados, lo que pone de manifiesto que las especies parecen ocupar distintos nichos biogeoquímicos en el ecosistema (Garten, 1978; Peñuelas et al., 2008). Tanto el tejido foliar como la hojarasca y el suelo asociados al quejigo tuvieron unos niveles de enriquecimiento en nutrientes superiores a los observados para el alcornoque.

Como se ha comentado anteriormente la calidad de la hojarasca, sus rasgos químicos y estructurales, determinan el proceso de descomposición (Pérez-Harguindeguy et al., 2000; Cornwell et al., 2008; Wieder et al., 2009). Los resultados presentados sugieren que en estos bosques la calidad del desfronde, en particular la concentración de calcio, es un factor determinante de la tasa de descomposición de la hojarasca durante la primera fase del proceso. En una segunda fase, en la que la tasa de descomposición se aproxima a cero, son las condiciones ambientales, especialmente el contenido de calcio del suelo, las que determinan el valor límite de la descomposición (Berg et al., 2003). Trabajos anteriores en sistemas forestales templados han mostrado que existe una relación significativa entre los niveles de calcio y la actividad descomponedora (Chadwick et al., 1998; Berg, 2000; Hobbie et al., 2006) ya que la microflora requiere de este elemento como cofactor de las enzimas que degradan la lignina y pectina (Eriksson et al., 1990; Berg et al., 2003). El análisis de la dinámica de la descomposición química mostró una acumulación neta de calcio en la hojarasca al comienzo del experimento, lo que sugiere que en estos bosques

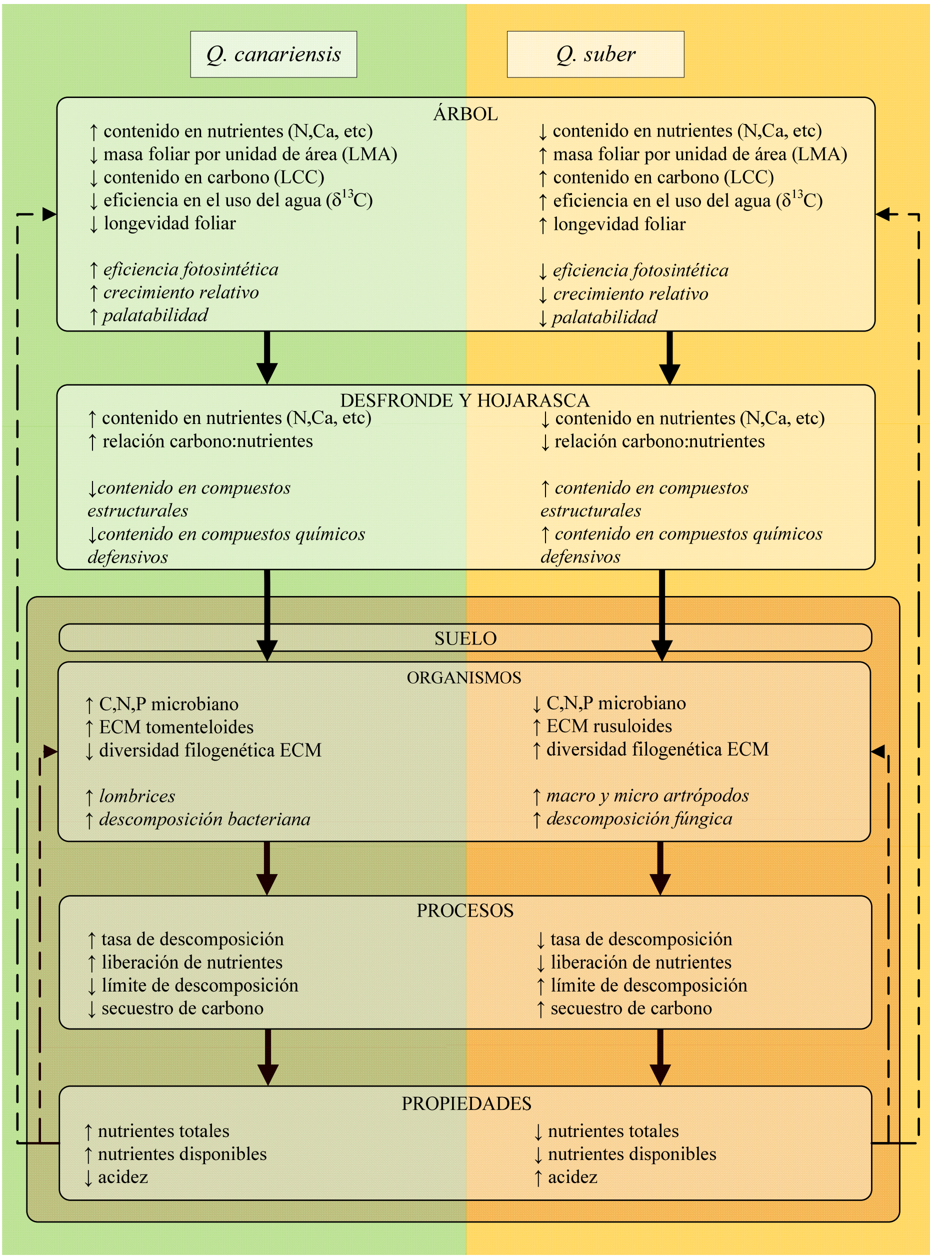

Figura 1. Las diferencias en los rasgos funcionales de las dos especies forestales, *Q. canariensis* y *Q. suber,* generan distintos efectos en el ecosistema. La calidad de los recursos producidos por ambas especies condiciona la composición de las comunidades del suelo que determinan los procesos de descomposición y mineralización, y generan potenciales procesos de retroalimentación (línea discontinua). En cursiva se incluye información obtenida de fuentes bibliográficas relativas a estas dos especies de quercíneas (Gallardo y Merino, 1993; Quero et al., 2006; Pérez-Ramos et al., 2010) o a la generalidad de especie arbóreas caducifolias y perennifolias (Antúnez et al., 2001; Wardle, 2002; Reich et al., 2005; Villar et al., 2006).

sobre suelos ácidos el calcio es un factor limitante para la descomposición (Swift et al., 1979; Staaf y Berg, 1982). Las diferencias en la concentración de componentes comúnmente relacionados con la tasa de descomposición (N, lignina) (Pérez-Harguindeguy et al., 2000; Sariyildiz y Anderson, 2003) que este y otros estudios han encontrado en las hojas de las dos especies estudiadas (Gallardo y Merino, 1993) no permiten explicar las diferencias observadas en las tasas de descomposición en estos bosques. Nuestra sugerencia es que el factor que controla la descomposición es aquél que es limitante en cada ecosistema (Hobbie y Vitousek, 2000; Kaspari et al., 2008; Hättenschwiler y Jørgensen, 2010)

La mayor calidad del desfronde del quejigo condiciona su mayor tasa de descomposición y genera como consecuencia una mayor disponibilidad de nutrientes en el suelo. La mayor concentración de nutrientes en el suelo se relaciona con un incremento de la fracción de hojarasca que llega a ser mineralizada y con ella, de nuevo, de la disponibilidad de nutrientes. Estas condiciones de mayor fertilidad asociadas al quejigo parecen generar un efecto de retroalimentación positivo sobre el proceso de descomposición. Por el contrario el alcornoque, cuya hoja tiene una menor calidad, se descompone más lentamente y libera menos nutrientes generando un ambiente menos fértil donde el proceso de descomposición se ralentiza y se alcanza antes el límite de la descomposición. Esto da lugar a un mayor acúmulo de materia orgánica y a un mayor secuestro de carbono en el suelo. El análisis de la dinámica de descomposición de los diferentes elementos permite observar cómo las diferencias entre especies generan un distinto retorno de nutrientes y ciclado de los elementos. La literatura recoge numerosos ejemplos en los que las especies que generan una hojarasca con un elevado contenido en nutrientes tienen una mayor tasa de descomposición e incorporan una menor fracción de materia orgánica al suelo mientras que aquéllas especies con hojarasca de baja calidad y elevada concentración en compuestos defensivos (p. ej. fenoles) favorecen la acumulación de C y N en sus suelos como resultado de una menor tasa de descomposición (Cebrián et al., 1998; Finzi et al., 1998b; Aber y Melillo, 2001; Berg et al., 2003; Hobbie et al., 2006).

Las diferencias entre rasgos foliares de las especies forestales, en este caso en el contenido en Ca, afectan al proceso de descomposición y mineralización y dan lugar a suelos más fértiles bajo la especie caducifolia mientras que los suelos son más pobres bajo la especie perennifolia. El efecto de las especies en los procesos del ecosistema es resultado, en último término, de su influencia en la comunidad de organismos del suelo que son los que controlan estos procesos (Wardle, 2002; Bardgett y Wardle, 2010). Los mayores valores de fertilidad encontrados en los suelos asociados a *Q. canariensis* comparados con los suelos bajo *Q. suber*, son resultado en parte de su distinta tasa de descomposición, y están relacionados con las propiedades de la masa microbiana. En general los valores de carbono, nitrógeno y fósforo microbiano fueron superiores bajo la especie caducifolia que bajo la perennifolia. Los análisis desvelaron que los valores de N y P microbiano se relacionaban positivamente con los de N y P inorgánico disponible y que en conjunto representaban una importante fracción del contenido total del suelo. Esto sugiere que la masa microbiana desempeña un papel clave en la mineralización y la regulación de nutrientes disponibles e indica la importancia del control de las especies sobre este componente del suelo.

Los resultados mostraron una importante variabilidad estacional en las propiedades microbianas (contenido de N y P) que condicionó las diferencias observadas entre micrositios. La estacionalidad afecta a las poblaciones microbianas a través de cambios en la humedad del suelo y mediante la distinta disponibilidad de sustrato relacionada con la fenología de las plantas (Rinnan et al., 2008). La variabilidad intraanual en la calidad y cantidad de desfronde de las especies pertenecientes a distinto grupos funcionales influye en la variabilidad de los organismos del suelo, los procesos de descomposición y las tasas de liberación de nutrientes al suelo (Wardle, 1992; Rapp et al., 1999; Andivia et al., 2009). Los aportes de hojarasca rica en nutrientes asociados a la fenología marcescente del quejigo se extienden desde otoño a primavera mientras que el alcornoque genera un aporte más constante de sustrato, pobre en nutrientes, a lo largo del año; esta fenología diferencial podría contribuir a la variabilidad estacional observada (Navarro et al., 2005). A pesar de que es probable que las especies que generan recursos de modo puntual ejerzan efectos temporales en el suelo significativamente importantes este tema ha sido poco explorado (Wardle, 2002; Eviner et al., 2006).

Como consecuencia de sus atributos ecofisiológicos característicos las especies de plantas influyen de forma diferente en la composición de las cadenas tróficas del suelo, siendo su hojarasca degradada de forma selectiva por determinados tipos de organismos, con importantes repercusiones en los procesos de descomposición y disponibilidad de nutrientes en el suelo (Negrete-Yankelevich et al., 2008). Trabajos anteriores demuestran que las especies que producen un desfronde de elevada calidad acogen mayores densidades y biomasa de microflora y fauna descomponedora, y generan un mayor consumo de hojarasca por parte de la fauna saprofita lo que repercute en toda la cadena trófica de la descomposición (Hansen, 1999; Hobbie et al., 2006; Wardle, 2006). Reich et al. (2005) relacionaron la mayor tasa de descomposición de especies cuya hojarasca contenía altos niveles de Ca con una preferencia de las lombrices de tierra por sustratos enriquecidos en este elemento, pues parece que el Ca desempeña un papel esencial en la activación de su sistema inmune (Opper et al., 2010). Especies arbóreas que generan sustratos ricos en nutrientes sostienen cadenas tróficas dominadas por bacterias, nematodos, protozoos y lombrices que se asocian a elevadas tasas de descomposición y poca acumulación de carbono en el suelo. En cambio, la hojarasca empobrecida se relaciona con cadenas tróficas compuestas principalmente por hongos y artrópodos que dan lugar a una descomposición más lenta, a suelos ácidos y pobres en nutrientes y a un mayor secuestro de carbono en los suelo (Wardle, 2002; Bardgett y Wardle, 2010).

En los sistemas forestales estudiados el calcio, tanto del desfronde como del suelo, aparece como un elemento relevante en el proceso de descomposición de la hojarasca lo que sugiere que este elemento puede determinar la composición de la comunidad de organismos descomponedores. El calcio tiene también un efecto significativo sobre la composición de la comunidad de organismos simbiontes. Los resultados obtenidos muestran que ambas especies de quercíneas ejercen un efecto indirecto sobre la comunidad de hongos ectomicorrícicos asociados a sus raíces a través de distintos niveles de Ca y acidez en la hojarasca y el suelo. La acidez del suelo afecta de un modo específico a la fructificación, crecimiento, colonización y a las capacidades enzimáticas de los hongos ectomicorrícicos, alterando las interacciones competitivas entre especies y la composición de la comunidad (McAfee y Fortin, 1987; Erland y Soderstrom, 1990; Agerer et al., 1998). El gradiente de fertilidad y acidez generado por *Q. canariensis* y *Q. suber* crea unas condiciones selectivas para las especies simbiontes que se asocia con un cambio en la composición específica y la estructura filogenética de la comunidad simbionte. Este cambio supone una pérdida de diversidad filogenética en ambientes menos ácidos y ricos en Ca donde se segregan las especies resupinadas tomenteloides que poseen cierta capacidad saprófita para compensar la menor dependencia que tiene la planta en el hongo simbionte para captar recursos (Kõljalg, 1996; Kõljalg et al., 2000). La mayor diversidad filogenética se encontró en las zonas más empobrecidas, bajo los alcornoques, donde abundaron las especies epigeas rusuloides. Los cambios en la composición de las comunidades ectomicorrícicas se han observado anteriormente como resultado de procesos de deposición de nitrógeno o en experimentos de fertilización relacionados con un incremento de la fertilidad del suelo (Peter et al., 2001; Lilleskov et al., 2002; Avis et al., 2008).

De nuestros resultados se desprende que el calcio desempeña un papel significativo en la dinámica biogeoquímica del ecosistema, pues se relaciona con la acidez de los suelos y la disponibilidad de nutrientes, el contenido en C y N del suelo, los procesos de descomposición y la estructura de la comunidad heterótrofa del suelo. Numerosos estudios de la última década demuestran la importancia del calcio y de las diferencias específicas en la concentración foliar de este elemento en el funcionamiento del ecosistema (Dijkstra, 2003; Fujinuma et al., 2005; Reich et al., 2005; Cornelissen et al., 2006; Hobbie et al., 2006). Sin embargo, dada la elevada correlación observada entre elementos químicos en el tejido foliar y en el suelo, es necesario ser cauteloso a la hora de imputar a un solo elemento un papel clave en el funcionamiento del ecosistema.

RETROALIMENTACIÓN Y DINÁMICA DEL ECOSISTEMA

En las interacciones planta-suelo ambos componentes ejercen una influencia sobre el otro. Cuando los cambios inducidos por la planta en las condiciones bióticas y abióticas del medio repercuten en la propia planta, ya sea de un modo positivo (p. ej. relaciones mutualistas) o negativo (p. ej. relaciones parasíticas), se produce un ciclo de retroalimentación (Binkley y Giardina, 1998; Catovsky y Bazzaz, 2000; Ehrenfeld et al., 2005).

Los resultados presentados muestran que el sistema forestal estudiado alberga distintos procesos mutualistas que dan lugar a potenciales ciclos de retroalimentación. Hemos observado que ambas especies de quercíneas, a través de sus distintos rasgos foliares influyen sobre la masa microbiana; a su vez, los microbios del suelo son directamente responsables de la mineralización de los nutrientes disponibles para la planta. La distinta calidad de la hojarasca tiene un efecto en el proceso de descomposición, posiblemente ligado a su efecto sobre los componentes de la cadena trófica del suelo, que favorece la liberación de nutrientes. De este modo las especies forestales modifican las condiciones de fertilidad del suelo en un sentido que parece favorecer a la propia especie "ingeniera", como se desprende del análisis de relaciones causales. Además, a través de los cambios en la fertilidad del suelo las especies influyen de modo indirecto en las comunidades de hongos micorrícicos con los que establecen relaciones mutualistas. De este modo se establecería un ciclo de retroalimentación en el que las especies forestales influyen sobre las comunidades de organismos del suelo y éstas a su vez afectan a las propias especies de árboles.

La covariación que habitualmente existe entre la distribución de las especies y determinados factores del medio, como por ejemplo la textura y profundidad de los suelos, impiden determinar con claridad el origen de estos ciclos de retroalimentación y por tanto, cuestionan su existencia. Nuestra hipótesis de que ambas especies de *Quercus* generan ciclos de retroalimentación positivos que favorecen su permanencia y coexistencia se sustenta en distintos resultados obtenidos. Las diferencias encontradas en los niveles de fertilidad del suelo y los valores de masa microbiana relacionados con las dos especies de *Quercus* se observaron con mayor intensidad en los horizontes superficiales que en los sub-superficiales lo que sugiere que el efecto de las dos especies se produce en forma de cascada (“top-down”) mediado por la distinta calidad de sus aportes al suelo. Por otro lado, la evaluación de múltiples modelos causales alternativos indicó que el modelo que mejor se ajustaba a los datos empíricos disponibles era el que asumía la existencia de un ciclo de retroalimentación por el cual las condiciones generadas por las especies repercutían positivamente en su propia distribución. Existen además evidencias de que ambas especies de quercíneas poseen un distinto nicho de regeneración; trabajos anteriores llevados a cabo en ésta y otras áreas de estudio próximas han demostrado que

Q. canariensis tiene un mayor éxito de reclutamiento cerca de adultos conespecíficos y en suelos más fértiles que *Q. suber*, que en cambio puede establecerse en zonas donde el aporte de nutrientes a través de la hojarasca es menor (Maltez-Mouro et al., 2005, 2009; Pérez-Ramos et al., 2010). Por último, el modelo de retroalimentación propuesto es acorde con los resultados de numerosos estudios recientes acerca de las características ecofisiológicas de las especies vegetales que difieren en rasgos funcionales, como la longevidad de la hoja, y de sus interacciones con las propiedades del suelo (Hobbie, 1992; Aerts, 1995; Knops, 1997; Cornelissen et al., 1999; Morris et al., 2008).

En conjunto, estos trabajos muestran que las especies caducifolias se caracterizan por tener un mayor área específica foliar (menor LMA, masa por unidad de área), una mayor concentración de N (relacionada con una mayor cantidad de rubisco) y una menor relación carbono:nutrientes. Las caducifolias tienden también a presentar una menor eficiencia del uso del agua. Dichas características confieren a las caducifolias una mayor capacidad fotosintética, que les permite sustentar un elevado crecimiento relativo, aunque también implican una mayor palatabilidad que supone una mayor pérdida de tejido por herbivoría. En cambio, las hojas de las especies perennifolias presentan hojas con mayor masa por unidad de área y mayor relación carbono: nutrientes, lo que limita sus tasas de crecimiento. Además, estas especies invierten una mayor cantidad de recursos en compuestos defensivos (estructurales o químicos) que reducen la palatabilidad y contribuyen a una mayor longevidad foliar. Ambos grupos funcionales muestran una distinta gestión de los recursos: las caducifolias poseen una estrategia adquisitiva pues debido a su funcionamiento demandan y pierden gran cantidad de recursos mientras que la estrategia conservativa de las perennifolias supone una menor demanda y pérdida de recursos. Estas diferencias afectan a las comunidades de organismos del suelo y a los procesos que éstos controlan. Así las características foliares de las caducifolias favorecen una mayor tasa de descomposición y liberación de nutrientes que dan lugar a ambientes más fértiles donde este grupo funcional tiene ventajas competitivas dada su mayor capacidad para captar recursos y limitar el acceso de otras especies a estos mismos recursos. Por el contrario, los rasgos foliares de las perennifolias ralentizan la descomposición y mineralización de la hojarasca, generando ambientes con menor disponibilidad de nutrientes donde este grupo es dominante gracias su uso más eficiente de los recursos. En suma, todo indica que el efecto que los rasgos funcionales de las dos especies, *Q. canariensis* y *Q. suber,* tiene en las comunidades del suelo genera distinta huella en los procesos biogeoquímicos, dando lugar a ciclos de de retroalimentación que confieren estabilidad al ecosistema y favorecen la coexistencia de ambas especies por una separación de nichos biogeoquímicos.

INTERACCIONES Y CAMBIO GLOBAL: LÍNEAS DE FUTURO

Los motores (*drivers*) del cambio global, como la alteración de los ciclos biogeoquímicos, el cambio climático y los cambios de uso del suelo, actúan simultáneamente modificando la composición y el funcionamiento de los ecosistemas terrestres. En la última década Sardans y Peñuelas (2004, 2005, 2007) han llevado a cabo varios experimentos de simulación de cambio climático en sistemas forestales mediterráneos. Sus resultados muestran cambios significativos en las concentraciones de nutrientes en el suelo y la vegetación así como en las actividades enzimáticas del suelo como consecuencia de una mayor aridez. La alteración global de los ciclos biogeoquímicos, los elevados niveles de CO_2 o la deposición de nitrógeno, pueden generar un desequilibrio estequiométrico y modificar el funcionamiento del ecosistema. El incremento de la temperatura y la disminución de la precipitación previstos en las zonas mediterráneas, así como los cambios de uso del suelo están provocando un cambio en la composición específica de las comunidades forestales, con una previsible mayor esclerofilización de la vegetación, que a su vez modificará las propiedades y procesos del ecosistema (Thuiller et al., 2005; Alexander y Arthur, 2010).

Los resultados de esta tesis suponen un avance en el conocimiento de las interacciones planta-suelo en bosques mediterráneos. Sin embargo, es necesaria una mayor comprensión de sus repercusiones sobre la dinámica forestal para poder generar modelos que contribuyan a preveer la respuesta de los ecosistemas ante el cambio global (Bardgett y Wardle, 2010). Para ello es indispensable comprender los mecanismos que generan los ciclos de retroalimentación. En las interacciones planta-suelo se han llevado a cabo muchos estudios sobre el efecto que las especies vegetales tienen en las propiedades bióticas y abióticas del suelo, sin embargo el número de trabajos centrados en cómo esas propiedades repercuten en las plantas es notablemente menor. Los estudios demográficos en campo complementados con trabajos experimentales que permitan diferenciar los efectos bióticos y abióticos del suelo sobre la planta (Kardol et al., 2007; Kulmatiski y Kardol, 2008) representan la mejor alternativa para comprender estas interacciones.

Otra aproximación recomendable es el uso de modelos espacialmente explícitos. El efecto de las especies forestales en los procesos del suelo es más aparente cuando las plantas que poseen una ecología contrastada ocurren en una misma comunidad pero están segregadas en el espacio. En estas circunstancias las plantas modifican directamente el suelo bajo su copa lo que permite distinguir un mosaico de propiedades en el suelo (Mlambo et al., 2007; Bennett et al., 2009). En cambio, en sistemas más densos donde se propicia la transferencia horizontal de la hojarasca, la huella de las especies se superpone. La mezcla de aportes de distinta calidad genera efectos que pueden no ser predecibles (Gartner y Cardon, 2004; Hui y Jackson, 2009). En nuestro trabajo hemos observado como en el bosque de La Sauceda donde la densidad de árboles es menor, las diferencias generadas por las especies de *Quercus* fueron mucho más intensas que en Tiradero, un bosque más denso, donde la superposición de las huellas de *Q. canariensis* y *Q. suber* atenuaron los efectos característicos de cada especie de modo que las diferencias llegaron a ser imperceptibles. Los modelos espacialmente explícitos son una herramienta de gran utilidad para modelar situaciones complejas en las que se superpongan las huellas de especies arbóreas y arbustivas.

El conocimiento del efecto del cambio global sobre las interacciones entre la comunidad forestal (sistema *aboveground*) y el suelo (sistema *belowground*) es un reto complejo pero necesario para diseñar planes de manejo que garanticen la provisión de bienes y servicios ecosistémicos, como la protección del suelo, el secuestro de carbono y el reciclado de nutrientes, por los bosques del futuro.

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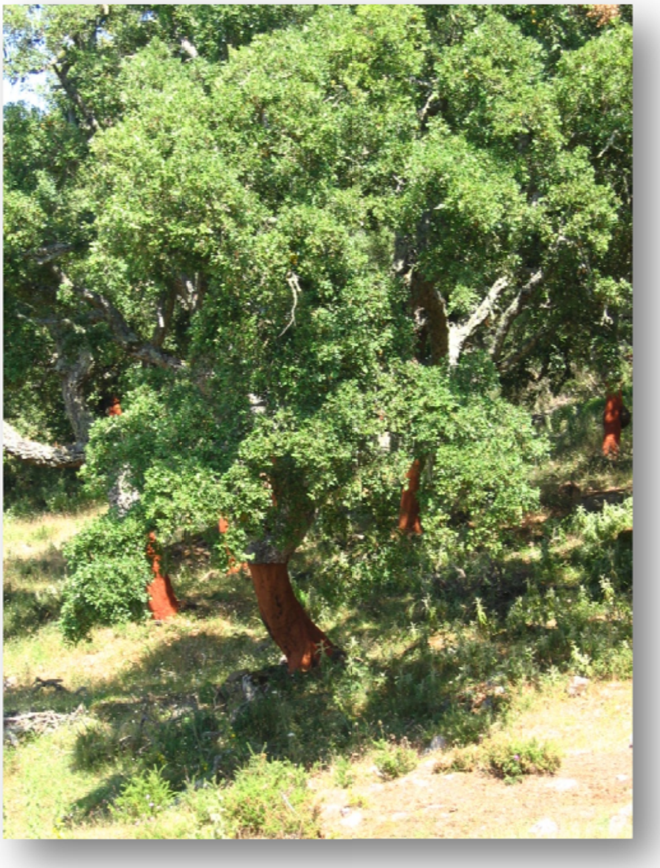
Capítulo 9.

Conclusiones



Capítulo 9. **Conclusiones**

1. La comunidad de especies leñosas mediterráneas estudiada mostró un amplio espectro de rasgos foliares. La variabilidad de los rasgos químicos fue, en general, superior a la de los rasgos morfológicos. Los contenidos de micronutrientes y elementos no esenciales fueron los atributos más variables entre las especies de la comunidad.

2. La discriminación isotópica foliar del nitrógeno ($\delta^{15}N$) reflejó el tipo de relación simbiótica o parasítica de la especie. La relación del $\delta^{15}N$ con otros rasgos funcionales a nivel de comunidad, como la masa foliar por unidad de área o la relación carbono: nitrógeno, señala su potencial como indicador de cambio en la composición funcional de la comunidad.

3. La relación encontrada entre la masa foliar por unidad de área (LMA) y otros rasgos foliares a escala de especie y de comunidad consolidan esta variable como un marcador funcional de la dinámica del ecosistema. Esta variable revela la estrategia ecofisiológica adquisitiva o conservadora de recursos de la especie, su capacidad para modular la entrada de recursos (luz, nutrientes) y sus efectos en los ciclos biogeoquímicos del ecosistema.

4. A nivel intraespecífico se observó una importante variabilidad en la concentración de los elementos químicos, siendo el N, P y S los elementos menos variables entre individuos de *Quercus canariensis* y *Q. suber*. El mismo patrón de variabilidad fue observado en todos los componentes del ecosistema estudiados: hoja viva, hoja de desfronde, hojarasca, suelo superficial y sub-superficial.

5. Se detectó un gradiente global de enriquecimiento en nutrientes, común para plantas y suelos del bosque, a lo largo del cual se diferenciaron los nichos biogeoquímicos de las dos especies de quercíneas. La especie marcescente *Q. canariensis* poseía tejidos más enriquecidos y ocupaba ambientes más fértiles que la perennifolia *Q. suber*.

6. El modelo teórico validado sugiere la existencia de un posible ciclo de retroalimentación en el que las dos especies de quercíneas, a través de un distinto retorno de nutrientes, modifican las propiedades del suelo de un modo tal que repercute positivamente sobre la presencia de la propia especie.

7. El proceso de descomposición de la hojarasca siguió una dinámica asintótica en la que se distingue una primera fase caracterizada por una tasa de descomposición (k_c) con valores próximos a 1.7 años^{-1} y una segunda

fase con una tasa de descomposición próxima a cero, en la que se alcanza el valor límite de la descomposición (C) alrededor de 0.4.

8. La calidad de la hojarasca y las condiciones ambientales generadas por ambas especies de quercíneas condicionaron el proceso de descomposición. El contenido de calcio en el desfronde fue la variable que mejor predijo la tasa de descomposición (k_c), mientras que el contenido en Ca en el suelo fue el mejor predictor del valor límite de la descomposición (C). Los contenidos de calcio en el suelo y en la hojarasca fueron también los mejores predictores de la tasa de pérdida de suelo forestal. La hojarasca enriquecida de *Q. canariensis* se descompuso más rápidamente (en cualquier ambiente), y las condiciones generadas bajo su copa favorecieron el proceso de descomposición de los dos tipos de hojarasca.

9. La mezcla de desfronde de ambas especies tuvo una respuesta no-aditiva. En las mezclas la descomposición de *Q. canariensis* se aceleró y la de *Q. suber* tendió a ralentizarse con respecto a los materiales no mezclados, indicando una posible interacción negativa. No se observaron relaciones de afinidad entre la hojarasca y la especie bajo la que se descomponía, rechazando la hipótesis de “la ventaja de jugar en casa” que sugiere que la hojarasca de una especie se descompone más rápidamente bajo el dosel de la especie que la produce.

10. La dinámica de liberación de los nutrientes de la hojarasca en descomposición fue muy variable dependiendo del elemento. Algunos (como el K) se liberaron rápidamente mientras que otros (como el Zn) mostraron una acumulación neta creciente. El Ca fue inmovilizado durante la primera etapa de la descomposición, posiblemente debido a la alta demanda por parte de los microorganismos descomponedores, y liberado en una etapa posterior. En general, la dinámica de liberación de elementos fue similar para las dos especies de quercíneas pero la liberación neta fue siempre superior para *Q. canariensis* debido a su mayor tasa de descomposición (k_c) y al mayor contenido inicial de nutrientes.

11. La masa microbiana de estos suelos forestales se encuentra influida por la estacionalidad, el tipo de cobertura vegetal y la profundidad del suelo. Se observaron cambios estacionales en el contenido microbiano de N y P, con valores superiores en primavera y otoño. Los valores registrados de las diferentes propiedades microbianas bajo *Q. canariensis* y bajo matorral fueron mayores que bajo *Q. suber* y en zonas abiertas. Estas diferencias sólo fueron perceptibles en primavera. Los valores microbianos de C, N y P decrecieron significativamente con la profundidad en todos los casos estudiados.

12. Asociada a las raíces de *Q. canariensis* y *Q. suber* se ha encontrado una diversa comunidad de hongos ectomicorrícicos dominada por tres familias: *Telephoraceae*, *Russulaceae* y *Cortinariaceae*. La distribución de las especies simbiontes estuvo condicionada por el gradiente de fertilidad y acidez del suelo generado por la distinta composición del desfronde de ambas especies. Dicho gradiente se asoció a cambios en la composición específica y en la diversidad filogenética de la comunidad micorrícica. Los suelos más fértiles, bajo *Q. canariensis*, albergaron una mayor proporción de especies resupinadas tomenteloides que los suelos más empobrecidos, bajo *Q. suber*, que acogieron una mayor diversidad filogenética dominada por especies rusuloides.