JOURNEY TO THE CENTER OF THE SOIL

Ecophysiological implications of soil compaction on plant development

David Alameda Márquez

TITULO: *Journey to the center of the soil. Ecophysiological implications of soil compaction on plant development*

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ISBN-13: 978-84-693-6388-1

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Prof. Dra. Carmen Galán Soldevilla, Directora del Departamento de Botánica, Ecología y Fisiología Vegetal, informa que el presente trabajo de investigación desarrollado por el Ldo. David Alameda Márquez bajo la supervisión del Prof. Dr. Rafael Villar Montero reúne todos los requisitos necesarios para aspirar al Título de Doctor por la Universidad de Córdoba.

Córdoba, 26 de abril de 2010

Carmen Galán Soldevilla — Rafael Villa

Carmen Galán Soldevilla — Rafael Villar Montero

Esta tesis doctoral ha sido realizada dentro de los proyectos DINAMED, Dinámica del bosque mediterráneo en un escenario de cambio global (CGL2005-05830-C03) e INTERBOS, Interacciones ecológicas y Cambio Global en el bosque mediterráneo (CGL2008-04503-C03-01). Plan Nacional de I+D+i Ministerio de Ciencia e Innovación del Gobierno de España.

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Cover design: Roots of *Cercis siliquastrum*
David Alameda Márquez

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VIAJE AL CENTRO DEL SUELO
Implicaciones ecofisiológicas de la compactación del suelo sobre el desarrollo vegetal

David Alameda Márquez

Abril 2010

A mi Madre y a mi Padre,
que juntaron tiempo y espacio para crearme.

Cuando la inclinación nos arrastra
hacia un conocimiento inconmesurable,
la sola existencia del límite
permite abarcar la inmensidad.

Ana Fernández-Reyes

Contents

Notes:
Chapters 2, 3, 5, and 6 include Spanish abstract.
Chapter 4 includes English abstract.
This current PhD Thesis is written in a bilingual format to aspirate to the European Mention.

Chapter 1

Introducción general

El suelo es el escenario donde se desarrollan gran parte de los acontecimientos de la vida terrestre. En él se encuentra un elevado porcentaje de toda la biodiversidad, es el lugar donde se desarrolla la rizosfera y con ella todas las plantas vasculares. El interés del ser humano en el estudio del suelo está ligado a su necesidad de entender cuáles son los procesos que lo constituyen y lo modifican, importante para el desarrollo de la agricultura y la ingeniería. El concepto histórico de "tierra" lleva implícito la importancia del suelo como sustento. Así, el nacimiento de la agricultura puede considerarse el momento en el que al suelo se le asigna una función vital en el desarrollo de las plantas.

El suelo se presentó desde los orígenes del ser humano como un mundo desconocido, idea que persiste hasta nuestros días al referirnos a los procesos que se dan en él como "una caja negra". Desde el nacimiento de las primeras actividades agrícolas se ha constatado el efecto positivo de remover o arar la tierra (Fig. 1). Sin saber cómo, nuestros ancestros intuyeron los efectos negativos que una mala estructura del suelo (compactación) tenía sobre el desarrollo de los cultivos. Actualmente sabemos que el arado tiene efectos positivos sobre los suelos de producción agrícola, debido a un incremento de la aireación, de la capacidad de infiltración y la retención de agua. Con ello se elimina gran parte de los efectos negativos de la compactación del suelo permitiendo el desarrollo óptimo de las raíces y con ello el crecimiento de las plantas.

Figura 1. Campesino arando. Pintura encontrada en la tumba de Sennedyem, ca. 1200 a. C., en Egipto (The Yorck Project, 2002).

El presente trabajo pretende evaluar la importancia de la compactación del suelo en el desarrollo vegetal usando como herramienta para ello la ecofisiología. Esta disciplina de síntesis tiene como propósito explicar la distribución y abundancia de las especies vegetales por medio del conocimiento de su fisiología (Reigosa y Pedrol, 2003). Así, se realiza una aproximación observacional desde las condiciones naturales en campo hasta aproximaciones experimentales en condiciones controladas, donde se aíslan los efectos fisiológicos de la compactación del suelo. Dicho recorrido en escalas se asemeja a un viaje: en él se parte buscando el patrón de la compactación en la naturaleza y los fenómenos que lo explican (Cap 2); continúa con la búsqueda de un modelo general de respuesta para las plantas (Cap 3); pasa por intentar explicar la interacción con otros factores de relevancia para el desarrollo vegetal (Cap 4 y 5); hasta llegar al centro mismo del suelo para desentrañar los mecanismos a nivel histológico que produce la compactación (Cap 6). Con todo ello se intenta describir el fenómeno de la compactación del suelo dentro del contexto de la ecología, analizando las implicaciones más relevantes que puede tener sobre el desarrollo vegetal.

Origen e interés de la compactación del suelo

La compactación del suelo se define como el incremento de la masa de suelo por unidad de volumen debido a la acción de una fuerza externa. De esta forma un suelo compactado ve modificada su estructura. Dado que la compactación es una variable "estructural" del suelo, es considerada un fenómeno multifactorial tanto en su origen como en los efectos que produce sobre la hidrología del suelo y el desarrollo de las plantas. A partir de los años 50 del siglo XX comenzó a ser estudiada como una variable que se presentaba comúnmente en los suelos de sistemas agroganaderos. La intensidad de pastoreo explicaba el aumento de la escorrentía debido a la formación de una capa compactada durante el pisoteo de animales (Alderfer & Robinson, 1947). El uso de maquinaría agrícola cada vez más extendido, se encontró como la causa principal de la disminución de la infiltración y el aumento de la erosión del suelo (Fullen, 1985). Los efectos negativos sobre la producción de cosechas de cereal hicieron de la compactación un tema de interés creciente que pronto se extendería a otros ámbitos de estudio. En el contexto forestal las perturbaciones provocadas por el empleo de maquinaria pesada en las labores de aclareo, extracción de madera, gestión contra incendios o durante las actividades de reforestación también producían compactación en el suelo (Greacen & Sands, 1980). La pérdida de cobertura vegetal, ya sea por largos periodos de sequía, incendios o el empleo de herbicidas favorecían la formación de suelos compactados, que constituye una de las causas más importantes en el lavado y empobrecimiento del suelo. La compactación entendida como resultado de perturbaciones, la convertirían en un indicador de calidad del suelo (Holland, 2004).

Los efectos de la compactación han sido muy estudiados en sistemas agrícolas y silvícolas, pero no se ha estudiado mucho desde un punto de vista ecológico. Según esta tradición, no es de extrañar que la mayor parte de los trabajos hayan sido publicados en la rama de "Soil Sciences" (Fig 2), y sólo una pequeña parte (alrededor de un 7%) en la categoría de "Ecology".

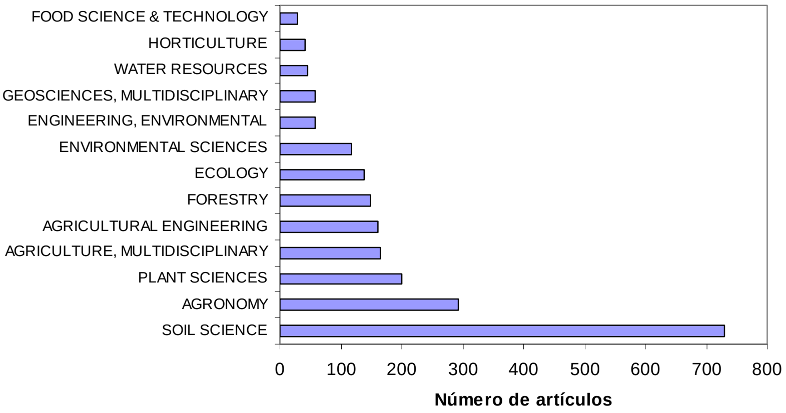


Figura 2. Número de artículos científicos en los que aparece el término "soil compaction". Busqueda en Science Citation Index (abril 2010). Total de articulos= 1860.

Sin embargo, los suelos naturales sin ningún tipo de perturbación también pueden mostrar un grado distinto de compactación (Pérez-Ramos, 2007; Quero et al., 2008; Gómez-Aparicio et al., 2008; Pérez-Ramos et al., 2010).

Las variables de la compactación

Para caracterizar un suelo compactado es necesario recurrir a variables indirectas que nos lo describan. En primer lugar el tamaño y proporción de las partículas de suelo es uno de los determinantes del grado de compactación. Así, la textura del suelo es una variable tenida en cuenta para describir los efectos potenciales de una perturbación. La humedad del suelo es el segundo factor de relevancia para este fenómeno. La cohesión de las partículas de un suelo así como su movilidad vienen determinadas por su grado de humedad.

Por otro lado, el contenido de materia orgánica es una variable a tener en cuenta en el proceso de compactación, dado que además de influir en el contenido de agua, participa en la formación de la estructura. Los procesos de humectación y desecación, producen cambios en la estructura, denominado bajo el nombre de histéresis, que pueden tener como resultado un aumento de la compactación. Estas tres variables textura, humedad y contenido de materia orgánica afectan de manera crucial al rango de compactación potencial que puede alcanzar un suelo.

Un suelo dada su constitución porosa tiene dos densidades: la real y la aparente. La primera es considerada la densidad de las partículas, valor dependiente del origen mineral y de la textura (Blanco-Sepúlveda, 2009). La densidad aparente, a diferencia de la real, es informativa del nivel de compactación de un suelo. Medida como la relación entre la masa de un suelo y su volumen (incluyendo los vacíos), describe la proporción de espacios porosos y partículas de suelo. Así, la porosidad también quedaría expresada de manera indirecta por la densidad aparente:

$$\boxed{P = \frac{\rho_r - \rho_a}{\rho_r} \times 100}$$

Siendo P la porosidad, ρ_r la densidad real y ρ_s la densidad aparente.

Otra variable que se usa normalmente para caracterizar la compactación del suelo es la dureza o resistencia a la penetración. También se denomina índice de cono, por ser medida como aproximación a la resistencia que ejerce el suelo a ser penetrado por un cono de dimensiones conocidas. La dureza del suelo es una variable de gran importancia biológica, al informar de la resistencia mecánica que tienen que vencer las raíces en su avance en el interior de un suelo.

Por último, una variable de gran importancia biológica afectada bajo compactación es potencial matricial del suelo. Éste nos informa acerca de la fuerza con la que el agua es retenida en el interior de la matriz de suelo. Al disminuir los espacios entre las partículas de un suelo, el agua es inmovilizada con mayor fuerza, de ahí que para extraer una cantidad de agua en un suelo compactado tengamos que ejercer mayor presión negativa (succión).

La compactación del suelo en el desarrollo vegetal

Como se ha explicado la compactación del suelo muestra una interacción compleja entre múltiples variables (textura, humedad del suelo, materia orgánica, etc.), por lo que presumiblemente la respuesta de las plantas a la compactación será también compleja. En general la compactación produce dos efectos fundamentales en el desarrollo vegetal: elevada impedancia mecánica sobre el crecimiento radicular y limitaciones en las relaciones planta-suelo, fundamentalmente en el proceso de toma de agua y nutrientes. Así, desde un punto de vista fisiológico la compactación del suelo va a provocar dos tipos de estrés, mecánico e hídrico. Como se explicará en el siguiente apartado, la limitación del crecimiento de la raíz ha sido ampliamente estudiada como el efecto más evidente sobre el desarrollo.

Las respuestas a nivel de planta completa indican una gran variabilidad de los efectos según el rango de compactación que consideremos (Alameda & Villar, 2008), el tipo de suelo (Whalley et al., 1995) y la especie (Godefroid & Koedam, 2004). En general, podemos señalar una reducción del crecimiento y una distorsión de la arquitectura de planta completa. Además se produce una limitación importante en los procesos de relación planta-suelo: disminución de la toma de agua y nutrientes, respiración radicular, pérdida de anclaje y dificultad para realizar simbiosis micorrícicas (Kozlowsky, 1999). La disminución del crecimiento puede explicarse como una respuesta plástica al estrés hídrico, donde pueden concurrir mecanismos tanto fisiológicos como morfológicos. Así, un mayor estrés hídrico conlleva el control de la apertura estomática que limita la asimilación de CO_2 por fotosíntesis (Conlin & van den Driessche, 1996). También, la disminución del crecimiento aéreo es el resultado de una aclimatación para reducir las demandas evaporativas, tanto por inhibición del crecimiento de las hojas como por el aumento de la abscisión (Kozlowski & Pallardy 1997). En cualquier caso, los fenómenos que observamos en la parte aérea son el resultado de lo que acontece en el contexto la raíz, donde se producen las señales de respuesta al estrés por compactación.

Rasgos radiculares modificados bajo compactación de suelo

Bajo los efectos de la compactación un suelo aumenta su dureza, su potencial matricial, disminuye los espacios porosos, y con ello la aireación y el almacenamiento de agua. En ese contexto son esperables respuestas de la raíz encaminadas a tolerar y/o evitar la impedancia mecánica, anaerobiosis y el estrés hídrico. La tasa de elongación de la raíz en condiciones de alta compactación se ve reducida como resultado de la impedancia mecánica (Barley, 1963; Taylor and Ratliff, 1969). Ante un obstáculo el crecimiento de la raíz responde de dos formas: intenta desplazarlo, o ante la imposibilidad de hacerlo, lo bordea. La respuesta inicial ante una limitación de la elongación es transmitida en forma de cambios a nivel anatómico, modificando el plano y la tasa de división celular (Dexter, 1987). El efecto es un aumento del diámetro radicular que puede interpretarse como un intento de aumentar la capacidad de penetrar en el suelo, disminuyendo la resistencia y la fricción sobre el ápice (Bengough et al., 1994, 1997, 2006). En el caso de no poder avanzar en la vertical, el giro de la trayectoria de crecimiento conlleva adaptarse a la matriz de espacios vacíos, adoptando la forma y tamaño de los poros por los que se avanza. Durante este proceso la producción de exudados con función lubricante (Cockcroft et al., 1967) y el consiguiente aumento de la presión osmótica, favorecen el avance disminuyendo la fricción. Es en la zona de elongación donde acontecen todos estos cambios plásticos, desde la anatomía a la arquitectura de la raíz.

Una raíz en un suelo no compactado lograría crecer en profundidad mediante la raíz principal, mientras que la exploración horizontal sería llevada a cabo a través de la emisión de raíces laterales o secundarias. Se ha demostrado que los cambios en la tasa de elongación llevan implícito una reducción de la tasa de producción de raíces laterales, dando lugar a un menor volumen de suelo explorado. La reducción de la longitud total de la raíz produce señales que limitan el crecimiento de la parte aérea con el objetivo de adaptarlo al abastecimiento hídrico que puede proveer la raíz. El etileno ha sido postulado como el regulador del crecimiento que es generado en la raíz y translocado hasta el tallo para inhibir le elongación del tallo y el crecimiento aéreo (Hussain et al., 2000). El resultado de esta comunicación raíz-porción aérea dará lugar a un patrón de crecimiento concreto en compromiso con otros factores ambientales: compactación, luz, disponibilidad de agua, etc. El resultado de dichas interacciones tendrá efectos importantes en el crecimiento y supervivencia de las plántulas, dependiendo de la estrategia seguida según la especie considerada.

La compactación en el contexto de la ecología

El éxito de una semilla al alcanzar un suelo vendrá determinado en gran parte por las características físicas de éste. La superficie del suelo debe ser capaz de suplir a la semilla de una humedad suficiente para permitir la germinación. Una vez comenzada ésta, la dureza, la disponibilidad de agua y nutrientes del suelo, garantizarán que a unas condiciones de luz adecuadas pueda emerger una plántula. Así, la compactación puede entenderse como un filtro inicial sobre el banco de semillas, limitando la implantación de las mismas y el consiguiente establecimiento de la plántula. Los efectos posteriores sobre el crecimiento de ésta vendrán determinados por la interacción con otros factores ambientales, tanto abióticos: luz, agua, temperatura, viento (estrés mecánico); como bióticos: competencia, herbivoría, etc. La respuesta plástica determinada por la especie conseguirá resolver el sentido de esas interacciones para alcanzar el estadío juvenil. Se han descrito efectos de la compactación del suelo visible en el desarrollo de juveniles de hasta 8 años (Gomez et al., 2002b). Sería esperable encontrar implicaciones en la *fitness* de un individuo sometido a condiciones de compactación intensa a lo largo de todo su desarrollo, probablemente medible en términos de capacidad competitiva, éxito reproductor o susceptibilidad a determinados factores ambientales ligados al desarrollo radicular, como por ejemplo, la sequía estival.

En los ecosistemas mediterráneos, donde se enmarca esta tesis, la marcada estacionalidad provoca cambios importantes en la estructura del suelo a la vez que restringe la disponibilidad de agua, limitando la supervivencia de las especies. La incidencia de plagas como el caso de "la seca" del encinar, se han relacionado con la presencia de zonas de alta compactación. El hongo fitopatógeno *Phytophtora cinamomi*, postulado como el agente causal de "la seca", requiere de alta humedad en suelo y de una mayor capacidad de retención de agua para prolongar su crecimiento. Las condiciones de alta compactación parecen ser apropiadas para la transmisión e incidencia de la enfermedad (Rhoades et al., 2003) siempre que exista una alta humedad en el suelo.

Por tanto, los episodios de sequía o la incidencia de plagas están siendo estudiados desde la perspectiva de la raíz, ya que entre ambos se produce un efecto sinérgico que intensifica el estrés hídrico.

Objetivos de esta tesis

Se pretende realizar una aproximación al fenómeno de la compactación desde diferentes perspectivas experimentales, centrándonos en 4 cuestiones fundamentales:

i) Búsqueda del patrón espacial de las variables de la compactación en condiciones naturales. Ha sido poco estudiado en situaciones de campo sin la influencia de perturbaciones, sin embargo, también existe una variabilidad espacial en la estructura del suelo. ¿Qué factores pueden explicar este patrón concreto?

Capítulo 2: Patrón espacial de la compactación del suelo y su relación con la cobertura vegetal: la huella del árbol sobre las propiedades físicas del suelo.

ii) Búsqueda de un modelo general de respuesta a la compactación. La idea general de que la compactación del suelo produce un efecto negativo sobre el crecimiento de las plantas es el resultado de aproximaciones experimentales donde ésta es usada de forma categórica y partiendo de valores elevados. Pero, ¿cuál es el modelo de respuesta a la compactación desde valores nulos en un rango continuo?

Capítulo 3: Compactación moderada del suelo: implicaciones sobre el crecimiento y la arquitectura de plántulas de 17 especies leñosas.

iii) Interacciones de la compactación con otros factores de relevancia para el desarrollo vegetal. Se sabe que la compactación modifica la arquitectura de las plantas. ¿Puede ser relevante en la respuesta frente a otras fuentes de estrés?

Capítulo 4: Compactación moderada del suelo: implicaciones en la respuesta a la sequía de 12 especies leñosas y el efecto de la lluvia estival.

Además, sabemos que la compactación no se da de manera aislada en la naturaleza. ¿Es independiente la respuesta a la compactación de otros factores ambientales? ¿Puede haber interacción, y por tanto, incrementar o disminuir sus efectos?

Capítulo 5: Respuesta de las plantas a la compactación bajo condiciones de sombra, bajo régimen hídrico y estrés mecánico – Un experimento con *Nicotiana tabacum*.

iv) Mecanismos de respuesta a la compactación. Los efectos más relevantes de la compactación se producen a nivel radicular y éstos son traducidos en cambios a nivel de crecimiento y funcionamiento de toda la planta. ¿Cuáles son los mecanismos de acción de la compactación del suelo? ¿Existe una secuencia temporal en sus efectos?

Capítulo 6: Relacionando rasgos radiculares con la fisiología y el crecimiento de planta completa de plántulas de *Fraxinus angustifolia* Vahl bajo condiciones de compactación del suelo.

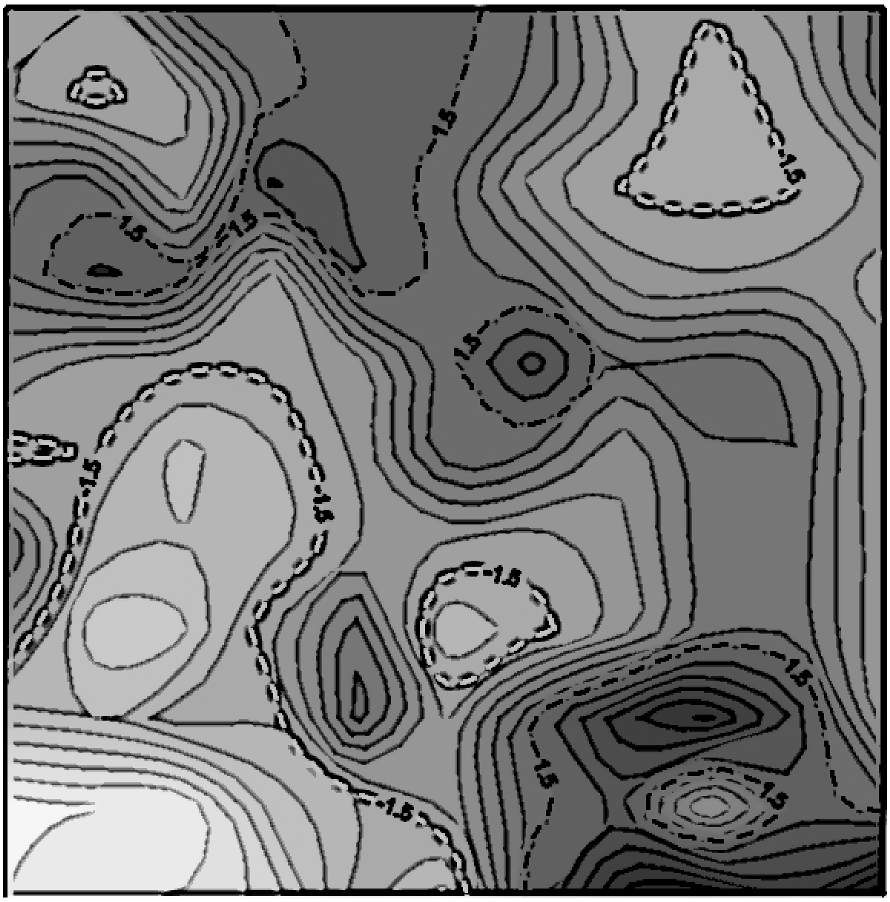
Aspectos novedosos de esta tesis

Este trabajo doctoral tiene ciertos aspectos que pueden ser considerados como novedosos dentro del contexto de estudio de las investigaciones de compactación de suelo:

1) Análisis de la compactación como variable continua. ¿Cómo estudiar la compactación? En general, los estudios se centran en realizar

tratamientos de tipo categórico, en el que se consideran 2 ó 3 niveles. Uno de los aspectos más interesantes es precisamente abordar el estudio de la compactación en un rango continuo y mediante aproximaciones estadísticas como las regresiones, en lugar de los análisis de la varianza, más adecuadas dado su gran poder predictivo, y su potencial al aplicarlos en modelos ecológicos (Cottingham et al., 2005). Así, hemos llegado a elucidar cómo es el modelo general de respuesta a la compactación.

2) Empleo de gran cantidad de especies silvestres**.** Existen pocos estudios que traten un amplio número de especies leñosas silvestres en condiciones experimentales. En la mayoría de los trabajos sobre especies leñosas se han realizado sobre especies de interés agrícola o forestal. Se ha completado así la información que tenemos sobre estas especies de interés ecológico en su potencial respuesta frente a las perturbaciones que provocan compactación.
3) Estudio de la compactación en campo sin perturbaciones y su distribución espacial. Existen pocos estudios en los que se evalúe la presencia y distribución de la compactación en zonas naturales, dado que el interés se ha centrado mayormente en analizar los efectos de la maquinaria agrícola y forestal. Se ha comprobado como existe un patrón espacial que está determinado por la cobertura arbórea.
4) Interacciones con otros factores de estrés. Raramente se ha estudiado el efecto de la compactación bajo la presencia de otros tipos de estrés. Hemos demostrado el alto compromiso que existe entre la compactación y otros factores ambientales.
5) Desde la histología a la fisiología vegetal**.** Se han analizado los efectos directos de la compactación sobre la histología y la morfología de la raíz, evaluando las consecuencias que tienen estos cambios sobre el funcionamiento de planta completa. Se postula un modelo conceptual de la secuencia temporal de efectos de la compactación y los mecanismos de respuesta por parte de las plantas.
6) Modelos conceptuales. Se han propuesto numerosos modelos generales que tratan de sintetizar y explicar los principales resultados.



Chapter 2

Spatial pattern of soil compaction and its relationship with plant cover: trees' footprint on soil physical properties

Partially published in:
Alameda, D., Villar, R. 2010. Patrón espacial de las variables de la compactación del suelo y su relación con la cobertura vegetal. En: II Libro de Ecología Espacial del Grupo de Trabajo de Ecología Espacial (ECESPA). M. De la Cruz (ed.). Madrid, España. (in press)

Abstract

Soil physical variables may exhibit a spatial structure which can inform us about soil genesis processes and soil-plant interactions. Analyzing that explicit pattern along the space we could rebuild which processes were involved within the soil structure formation and which role vegetation played in it. This work pays special attention to the influence of trees cover (*Quercus ilex* subsp. *ballota* and *Pinus pinaster*) in the spatial distribution of variables related to soil compaction. The spatial structure was analyzed by SADIE. Our results showed that variables related to soil compaction such as bulk density, penetration resistance, water content and organic matter showed an aggregate spatial pattern on nature despite no disturbance process being produced. Interestingly, the effect of the tree cover depends on the species. Thus, under *Q. ilex* cover there was a higher organic matter and a lower bulk density; on the contrary, *P. pinaster* cover produced the opposite effects. Soil compaction pattern and trees canopy had a clear effect on grass production. Grass production in 2007 and 2008 was higher in those microsites with lower compaction values (low bulk density and penetration resistance, and high organic matter content) and upon *Q. ilex* canopy; however grass productivity was reduced by *P. pinaster'* cover. Finally, a structural equation model is presented attempting to describe causal relation between tree cover, soil compaction and grass production. Due to this work was made to test spatial distribution of soil compaction variables, SEM model was built taking into account the spatial pattern obtained by cluster index of SADIE. Troubles about spatial autocorrelation are discussed.

Keywords: autocorrelation, bulk density, grass production, *Pinus pinaster*, *Quercus ilex*, SADIE analysis, SEM structural equation modelling, spatial association.

Resumen

Las propiedades físicas del suelo pueden mostrar una estructura espacial que nos informa de los procesos formadores del suelo y de la interacción suelo-planta. Analizando este patrón espacialmente explícito podemos reconstruir qué procesos concretos participaron en la formación de la estructura del suelo y qué papel jugó la vegetación. Este trabajo presta especial atención a la influencia de la cobertura de árboles (*Quercus ilex* subsp. *ballota* y *Pinus pinaster*) en la distribución espacial de las variables relacionadas con la compactación. Mediante el análisis espacial llevado a cabo con SADIE, se muestra cómo las variables de la compactación tales como densidad aparente, resistencia a la penetración, contenido de agua y materia orgánica, muestran un patrón agregado en la naturaleza sin que ninguna perturbación haya sido producida. Resulta llamativo como el efecto de la cobertura arbórea es dependiente de la especie que consideremos. Así, bajo *Q. ilex* hubo mayor contenido de materia orgánica y menor densidad aparente, mientras que la cobertura de *P. pinaster* produjo los efectos opuestos de manera significativa. El patrón de la compactación del suelo y la cobertura de árboles mostró tener un claro efecto sobre la producción de herbáceas. Así, para los años 2007 y 2008, la producción de pasto fue mayor en aquellos micrositios con baja compactación (baja densidad aparente y resistencia a la penetración y alto contenido de materia orgánica) y bajo encina: sin embargo, la producción fue significativamente menor bajo la cobertura de pino piñonero. Para concluir se realizó un modelo de ecuaciones estructurales (SEM) con el que se pretende describir las relaciones causales entre la cobertura de árboles, la compactación del suelo y la producción de pasto. Dado que este trabajo se llevó a cabo con el objetivo de analizar la distribución espacial de la compactación, el modelo SEM se realizó teniendo en cuenta el patrón espacial dado por los índices de agregación de SADIE. Se discuten los problemas derivados de la autocorrelación espacial de los datos.

Palabras clave: *autocorrelación, densidad aparente, Pinus pinaster, producción de pasto, Quercus ilex, SADIE, SEM structural equation modelling.*

Introduction

Physical properties of soils such as texture, porosity or structure are important from a biological point of view due to their role in water and nutrient uptake by plants. One variable that is highly related to these physical properties is soil compaction. Usually, compaction is defined as result of a soil disturbance such as machinery use in agricultural or forestry management or by animal trampling. In agricultural systems, compaction is found forming by a subsoil layer or horizontal patches associated with the tillage practice (Coelho et al., 2000). In forestry systems it is mainly related to silvicultural treatments (Godefroid and Koedam, 2004 and 2008) such as logging effects on timber extraction or machinery and human paths. Animal grazing is also another source of soil compaction as it has been found in grasslands (Drewry et al., 2008). However, apart from this origin mainly linked to human activity, there are natural factors which produce spatial patterns of soil compaction such as runoff, drought or fire (Kozlowsky, 1999).

Soil compaction effects on plant growth are highly dependent on soil type (Whalley et al., 2008), compaction range and the species studied (Godefroid and Koedam, 2004; Alameda and Villar, 2009). In general, compaction limits roots growth and subsequently this affects all the processes mediated by roots: anchorage, water and nutrients uptake. An important side effect of this root distortion is the above growth reduction, highly reported in literature about crop agriculture (Wolkowsky, 1990; Unger and Kaspar, 1994).

Compaction is generally measured as bulk density or penetration resistance. Bulk density is defined as soil dry mass per unit volume, which is related with soil structure. Penetration resistance measures as the force needed by roots to separate the soil particles, measured as approximation to the force applied by a cone probe. Many studies on soil compaction emphasise its multifactor character, because it resulted in the sharp interaction of different soil variables. Thus, granulometry or texture specify what size the particles and proportions are of each size class; bulk density tells us about the amount of particles per unit volume; water content is related to particles mobility; and organic matter content mediates the particle-particle connection. Moreover, it is possible to measure the force needed to separate this structure throughout the penetration resistance measurement which describe the soil strength. Thus, soil compaction may be understood as a complex of physical and mechanical variables interacting.

It is well known how trees growth modifies soil properties from seedlings to adult stage (Gómez-Aparicio et al., 2004). In this sense, soil can be considered a huge desert where life is discretely distributed, even more when microorganisms tend to aggregate in the space forming hot spots of activity (Hisssinger et al., 2009; Ranjard and Richaume, 2001). Rhizosphere is one of the most important hot spots of activity, due to plant's ability to create around the roots a specific micro-ambient to optimize their relation with soil and edaphic biota. These roots-mediated changes within the soil are connected with the upper part of the plant through tree canopy effects. Trees have been able to create a recycling system where nutrients are taken out from the soil and returned again into the soil by fallen leaves. The effect of tree canopy on soil properties is highly dependent on the species, mainly related to leaf habit and leaf chemical and physical traits. Thus, Shukla et al. (2006) found that under *Quercus gabelli's* canopy there was higher litter deposition and subsequently higher soil organic matter content and nitrogen than under *Pinus edulis*. Leaf chemical traits such as lignin content and tannins concentration (Nicolai, 1988), toughness (Gallardo and Merino, 1993) or physical barriers are important quality traits affecting decomposition and mineralization rates (Cornelissen, 1996). Therefore, the effects of tree canopy are dependent on species.

Despite soil chemical changes induced by litter input and decomposition, trees may also affect soil physical properties which could modify soil-water-roots relations. Thus, Bhojvaid and Timmer (1998) found how bulk density (apart from other soil chemical properties) is ameliorated under *Prosopis'* canopy. According to all the previously mentioned, trees have been considered as ecosystem engineer, modifiying soil and life around its influence.

In the Iberian Peninsula, most of the original *Quercus* forest has been transformed into savannah-like ecosystems called "*dehesas*". They are made by selective clearcut in order to increase grass productivity by diminishing trees density. *Dehesas* are characterized by their high production and grass species diversity, very becoming for cattle raising, balanced and sustainable. Common species in this ecosystem are *Quercus ilex*, principally, and others such as *Q. suber*, *Q. faginea* and *Q. pyrenaica*. In the Iberian Peninsula, *dehesa* is one of the most extensive systems together with pine forests. During the 1950s to 1970s in Spain, pine plantations were made in order to enhance economic activity in rural zones and to increase timber production (Quero et al., 2007). However, nowadays these forests are unmanaged due to the fall in wood prices and the high costs of timber extraction. From an environmental point of view, pine forests present several inconveniences because of their high fire risk and the scarce or even null grass productivity (Quero and Villar, 2009). Apart from the different origin of *Quercus* (natural) and *Pinus* (naturalized), do they have a different role as soil-engineer of *dehesa* ecosystem?

This work attempts to explain: (i) the existence of a spatial pattern (on surface and in depth) of soil compaction under "non-disturbing" conditions; (ii) which biotic or abiotic factor are involved in the formation of this spatial pattern and (iii) what role is tree canopy on soil compaction variables. Finally, we want to expound a model trying to explain the effects of the tree canopy on soil compaction variables and on grass production.

Material and methods

Site description

The study site was located at the Natural Park of Sierra de Cardeña y Montoro (Córdoba, Spain) (38° 14' 9'' N, 4° 21' 55'' W) within a fenced area to exclude ungulates hervibory (Fig. 1). Tree vegetation was formed by *Q. ilex* L. subsp. *ballota* (Desf.) and *Pinus pinaster* Aiton. Pines proceed of a 50 year old plantation. Shrub vegetation was scarce, only *Cistus ladanifer* L. and *C. albidus* L were found.

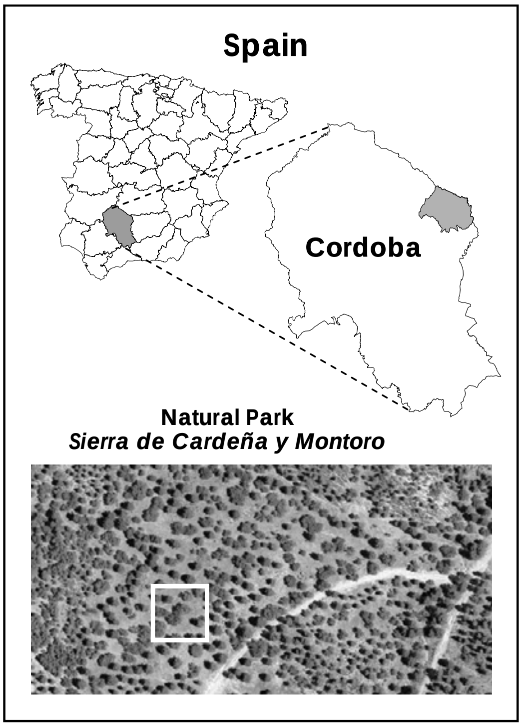


Figure 1. Location of studied area. The position of the "Sierra de Cardeña y Montoro Nature Park" can be seen, and an overview of the *dehesa* ecosystem where the grid of this study is found.

This area was at least 10 years of non-management and ungulates herbivory. The study was carried out over a square plot (40 x 40 m) with four-metre resolution. Soil and herbaceous sampling was taken every four metres for a total of 121 sampling points during the spring of 2008.

Soil measurements

Soil sampling was done at two depths (2-7 cm and 9-14 cm) in order to analyze the spatial pattern of soil physical properties on surface and in depth. Bulk density (BD) measurements were made with a metallic cylinder of 5 cm in height and 5 cm in diameter. Samples of first depth (2-7 cm) were taken discarding 2 cm from above to avoid litter in the sample and 2 cm below so as not to affect the soil structure of second depth (9-14 cm). Due bulk density has to be measured at soil field capacity, the sampling was made after a spring rainfall (28th of April 2008). To avoid water loss during sampling and transport from field to lab, each cylinder was sealed with plastic film. Soil mass water content (MWC) was calculated as the difference between wet, measured the day after sampling, and dry weight, obtained by drying all the samples by mean of an oven at 105 °C until constant weight. Bulk density was calculated as the ratio between dry soil mass and soil volume. Soil organic matter was measured by ignition in a muffla oven at 550 during 5 hours, knowing the total absence of carbonates. Three measures of penetration resistance were taken per sample point by means of a penetrologger (Eijkelcamp, The Netherlands) with 12.6 mm diameter and 30° cone angle, taking force measures each centimetre up to a total of 15 cm in depth. The three measurements were taken about 5 -10 cm around the soil sample. We calculate the mean value of the three measurements of penetration along the profile 2-7 and 9-14 cm.

Plant cover and grass production

Tree cover was estimated by counting (0, absence and 1, presence) through the lineal intersection method between an imaginary vertical line and each sampling point. Which tree species was above each point was taken into account (*Q. ilex* or *P. pinaster*). In July of 2007 and 2008 aboveground grass biomass was harvested into a square of 25 x 25 cm around each sampling point. Grass was dried at 70 ° C during at least two days to estimate grass production in dry biomass values.

Spatial analysis

Spatial pattern of soil compaction variables was analyzed by SADIE: Spatial Analysis by Distance IndicEs (Perry, 1998). SADIE analysis was carried out by "freeware" SadieShell v1.3 available in www.rothamsted.ac.uk/pie/sadie. This analysis technique is based on indices which quantify spatial pattern of a variable in terms of distance to regularity. Thus, SADIE computes two principal indices: index of aggregation (I_a) and clustering index (v). I_a provides information on the overall spatial pattern of the variable analyzed. When $I_a < 1$, it is assumed that the variable studied follow a regular pattern; if $I_a = 1$ a random pattern; and if $I_a > 1$ an aggregated pattern. The clustering index, v, quantifies the partial contribution of each sampling unit to the overall spatial pattern of the data. Thus, a v index is generated for each sample point in a continuous scale which allows plotting them in a contoured map through an interpolation technique. In our study maps were made by krigging interpolation using SURFER v8. (Golden Software Inc., Boulder, Colorado, USA). Each map shows patches and gaps. The patches are places with high values of the variable in terms of significant spatial aggregation ($v > 1.5$, named v_i by convention) and they are drawn with a colour range from grey to black. On the contrary, gaps are places with low values of the variable studied ($v < -1.5$, named v_j by convention) and they are drawn with a colour range from grey to white.

In order to know if the different variables coincided in their spatial aggregation pattern, a covariation spatial analysis was made. SADIE computes an index of overall association (X, chi-p) ranged between -1 (variables spatially disassociated) and +1 (variables spatially associated). At the same time a local association index (χ) is calculated to measure the partial contribution of each data to the overall association pattern. Significant values (*P*) for the SADIE indices (I_a , v_i , v_j , χ) are derived from a randomization test of 5967 permutations (see Perry, 1998).

It is important to remark how SADIE transforms each variable into a standardized value (v_i , v_j , χ) containing information about spatial distribution of the data. For instance, tree cover was taken as categorical variable (0 and 1) which is transformed into a continuous variable (-6 to +6), as clustering index. The high correspondence between the actual value of the variable and its cluster index is shown in Fig. 2 where it can be seen how tree cover is transformed into a map of gap and patches representing each tree species.

ANOVA analyses

A factorial ANOVA (analysis of variance) was made to test significant differences of soil compaction variables as function of cover type (below *Q. ilex*, below *P. pinaster* and open sites) and sampling depths (2-7 and 9-14 cm). The same procedure was used to test differences between grass production and cover type and sampling year. Analyses were made with Statistica 7.1 (StatSoft, Inc.).

Structural equation model

Structural Equation Modeling (Wright 1920, 1934) allows the exploration of the causal relationships between a set of variables according to a particular working model (Mitchell, 1996). This model is firstly a result of conceptual hypotheses attempting to explain how variables are interacting. Then they are translated into a path diagram where arrows indicate which of the many potential variables are explaining changes on a response variable. Although performing similar procedures rather than multiple regression analysis, SEM adds a goodness of fit of the model to the data (Mitchell, 1992; Austin, 2007). The model tested in our study was made attempting to explain the causal relations of tree cover on soil compaction variables and herbaceous productivity in the studied area.

The cluster index (*v*) for each variable was used instead of actual values due to our objective being to relate these variables along the space. Two problems can be detected in this approach: one is the "trouble" of spatial autocorrelation; the other one is related to the standardization procedure behind the cluster index calculation. About spatial autocorrelation Legendre (1991) addressed that "spatial heterogeneity is functional in an ecosystem and not the result of some random, noise-generating process". Thus, in ecological studies focused on the spatial heterogeneity and its similarity a mechanistic model, spatial autocorrelation has to be measured in order to use it in a conceptual or statistical model. To do this, Moran's coefficient was calculated for each variable into the sampling scale. Thus, the spatial autocorrelation was found for each variable between 4-40 m of sampling scale. Due to autocorrelation violates independency principle, the resultant model only can be used to explain the spatial causal relation in our studied area. With respect to the SADIE standardization process to calculate the cluster index, there is a remarkable question: cluster index presents a smoothing of the differences between values inside a patch or a gap, maximizing differences between both. Due to SADIE imposing a restrictive significant value to establish the existence of a patch or a gap, this standardization can be used in such as a way to reflect spatial aggregation of a variable though its cluster index (see Fig. 2). Thus, Maestre et al. (2003) used cluster index in a PCA analysis in order to translate the spatial patterns into a multivariate analysis to find out the relationship between their studied variables. In our work, we attempt to go a step further, translating spatial pattern (with unsolved autocorrelation) into a structural equation model, assuming and warning the potential bias of the extrapolation of the model. The structure of the hypothesized causal relationships between selected variables was chosen as a function of the highest statistical support, according to the significance of χ^2 and two indices of goodness of fit: NFI (Bentler and Bonett's Normed-Fit Index) and GFI (Goodness of Fit Index) (see Bolen 1989). χ^2 must be non-significant (P>0.05) indicating that the pattern of covariance predicted by the model is not distinguishable from the observed (Hayduk, 1987). On the other hand NFI and GFI as goodness of indices have to be greater than 0.9 indicating an acceptable fit of the model to the data (Bollen & Long, 1993). A first screening was made to choose which variables will fit into the model. A conceptual path-diagram was drawn taking into account those variables highly related to each other. Seven variables were put into the model: herbaceous production, penetration resistance, mass water content, bulk density, soil organic matter and *Quercus ilex* and *Pinus pinaster* cover. One model was performed at each depth, attempting to see how the causal relation changes or not as a function of depth.

Results

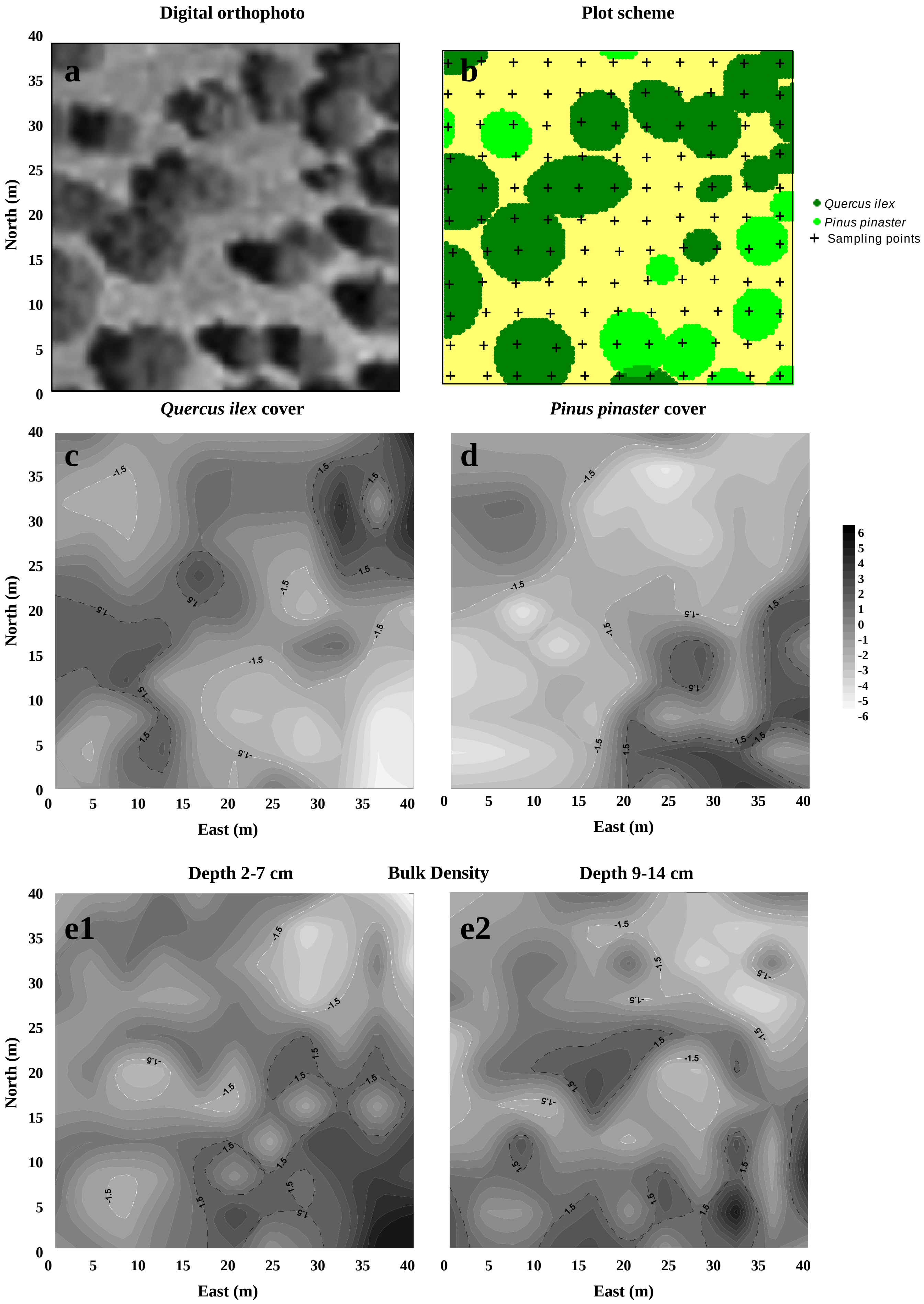
Spatial pattern of soil compaction

Bulk density, gravimetric water content, organic matter, and penetration resistance showed an aggregated pattern at 2-7 cm and 9-14 cm of depth (Table 1, Fig. 3). The only exception was organic matter at 9-14 cm of depth which seems to be closed to a random pattern (Fig. 3 g2). An aggregated spatial pattern determines patches (high values of the variables) and gaps (low values) (Fig. 3).

Table 1. Results of SADIE analysis and descriptive statistics to compaction variables. *P* values are derived from a randomization test (5967 permutations). I_a, aggregation index.

		I_a	P_a	N	Mean	SD	Max	Min
Bulk density (g cm^{-3})	2- 7cm	1.57	0.003	121	1.41	0.11	1.65	1.08
	9- 14 cm	1.77	0.000	121	1.46	0.10	1.72	1.18
Mass water content (%)	2- 7cm	1.52	0.006	121	10.40	4.35	52.46	5.44
	9- 14 cm	1.30	0.059	121	9.71	4.13	51.31	5.47
Organic matter (%)	2- 7cm	1.61	0.003	118	2.60	0.96	6.16	0.34
	9- 14 cm	0.99	0.457	121	2.20	0.75	7.68	0.82
Penetration resistance (MPa)	2- 7cm	2.22	0.000	121	1.49	0.66	4.54	0.50
	9- 14 cm	2.11	0.000	121	2.51	1.16	5.78	0.69

In general, all the variables measured in the first depth were positively associated with the value of the second depth (Table 2). Again, the exception was the organic matter which did not show continuity along depth. Bulk density was dissociated with organic matter in each depth (Table 2).


Digital orthophoto

Plot scheme

Quercus ilex cover

Pinus pinaster cover

Depth 2-7 cm

Bulk Density

Depth 9-14 cm

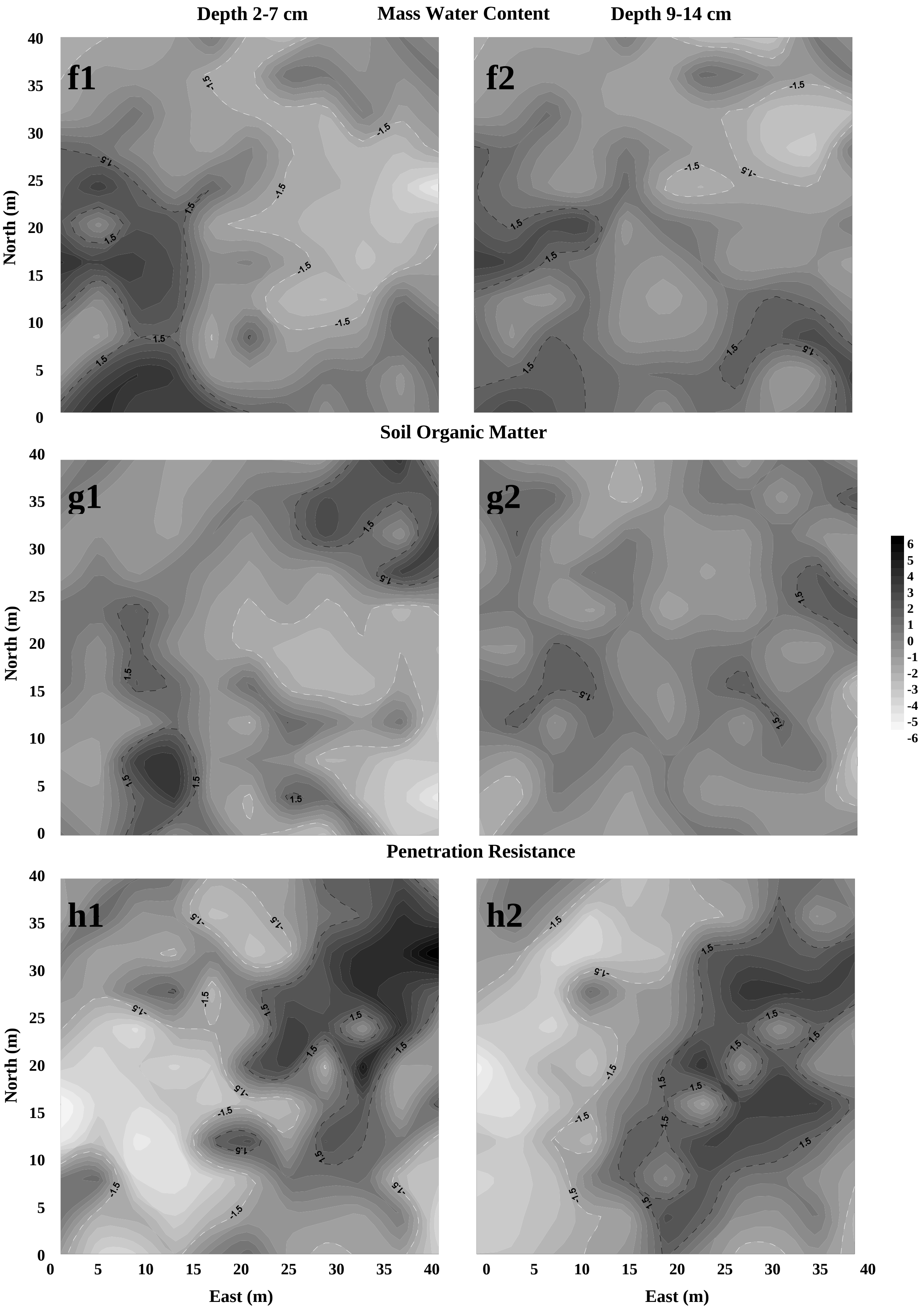

Depth 2-7 cm
Mass Water Content
Depth 9-14 cm
f1
f2
Soil Organic Matter
g1
g2
Penetration Resistance
h1
h2
North (m)
East (m)
0
5
10
15
20
25
30
35
40
1.5
-1.5
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6

Figure 2 (Panels a-d). Maps of the sampling area: a: Digital orthophoto showing the actual positions of tree cover; b: Plot scheme showing sampling points and the tree cover estimated by counting; c: Map of SADIE index of clustering for *Quercus ilex* cover; d: Map of SADIE index of clustering for *Pinus pinaster* cover. See the agreement between actual position and the interpolated position. Key scale has no units. See test for details.

Figure 3 (Panels e-h). Maps of SADIE index of clustering for soil compaction variables. Panels at left column show analysis for first depth (2-7 cm); to the right panels for second depth (9-14 cm). e: bulk density. f: mass water content. g: soil organic matter. h: penetration resistance. Key scale has no units. See test for details.

Penetration resistance was dissociated with gravimetric water content at both sampling depths (Table 2).

Table 2. SADIE association analysis between spatial pattern of compaction variables (n=121). Significant interaction, using p-level corrected by Bonferroni (P<0.001), are shown in bold text.

		Bulk density		**Mass water content**		**Organic matter**		**Penetration resistance**	
	Sampling depth	2- 7cm	9- 14 cm	2- 7cm	9- 14 cm	2- 7cm	9- 14 cm	2- 7cm	9- 14 cm
Bulk density	2- 7cm		**0.340**	-0.072	0.177	**-0.595**	-0.231	-0.122	-0.042
	9- 14 cm			0.175	**0.244**	**-0.293**	**-0.410**	**-0.278**	-0.188
Mass water content	2- 7cm				**0.638**	**0.282**	-0.003	**-0.503**	**-0.540**
	9- 14 cm					-0.026	0.093	**-0.505**	**-0.456**
Organic matter	2- 7cm						0.188	0.018	0.007
	9- 14 cm							-0.010	0.084
Penetration resistance	2- 7cm								**0.645**
	9- 14 cm								

Tree canopy effects

Soil compaction variables were slightly linked to plant cover. Thus, bulk density in the first depth was lower under *Q. ilex* canopy than under *P. pinaster* and open sites (Fig 3). However, bulk density at the second depth was similar for the different cover types. Gravimetric water content for both depths was lower in open sites than in *Q. ilex* and *P. pinaster* canopies. Organic matter showed the same differences between sites that bulk density. Only sites covered with *Q. ilex* showed a higher organic matter than the other cover types (but only at first depth). Penetration resistance was lower at first depth than second one, but similar penetration resistance values were found between cover types at both depths (Fig 3).

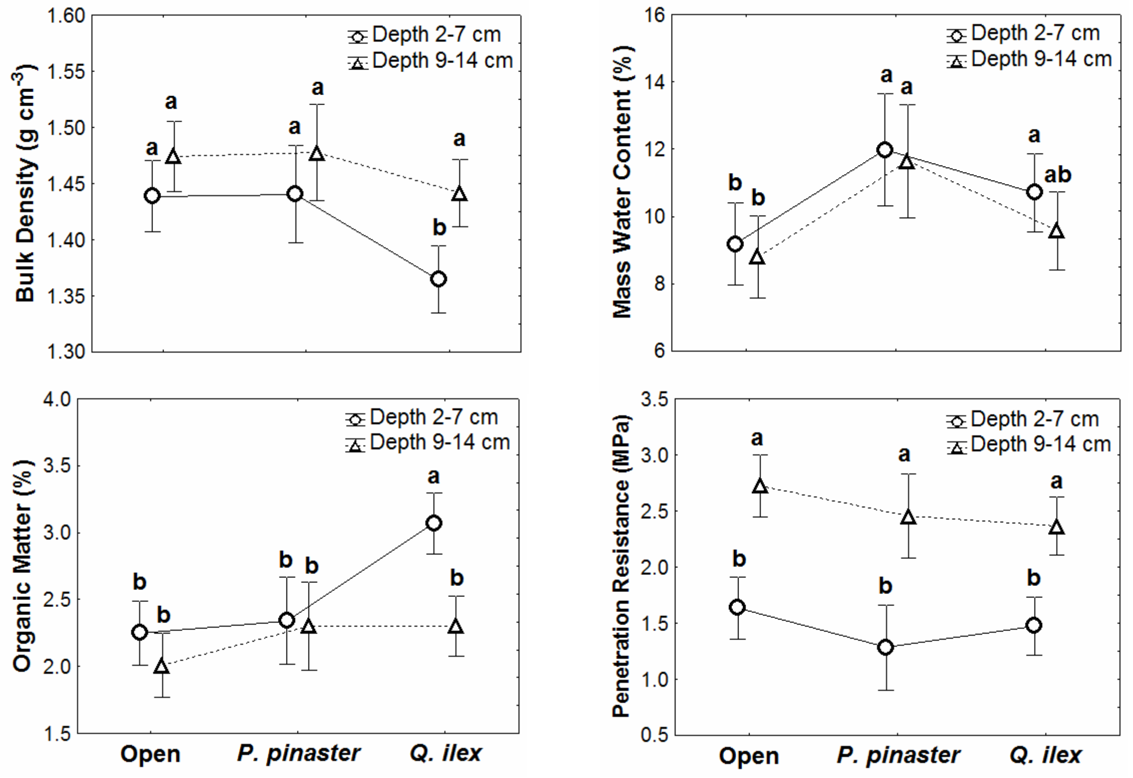


Figure 3. Results of ANOVA analysis for compaction variables in function of cover type. Significative differences are shown with different letters.

SADIE covariation analysis showed that soil compaction variables were related to plant cover. However, the effect was mainly found at the first depth (2-7 cm) (Table 3). In general, under *P. pinaster* canopy and open sites there was a high bulk density values and lower organic matter content. *Q. ilex* sites exhibited the opposite situation: low bulk density and high organic matter content. However, mass water content and penetration resistance did not show any association with cover types.

Table 3. SADIE association tests between spatial pattern of compaction variables and spatial pattern of cover type. Χ (chi-p) is the index of overall association at P probability level. Associations are significant when *P*<0.025; dissociations are significant when P>0.975. Significant results are shown in bold text.

		Bulk density		**Mass water content**		**Organic matter**		**Penetration resistance**	
		2- 7cm	9- 14 cm	2- 7cm	9- 14 cm	2- 7cm	9- 14 cm	2- 7cm	9- 14 cm
Pinus pinaster	χ	**0.405**	0.186	-0.165	0.018	**-0.311**	-0.045	0.145	-0.022
	P	0.000	0.032	0.955	0.436	1.000	0.675	0.079	0.587
Quercus ilex* subsp. *ballota	χ	**-0.671**	**-0.346**	0.076	-0.157	**0.491**	**0.256**	-0.010	0.106
	P	1.000	1.000	0.217	0.942	0.000	0.003	0.539	0.124
Open sites	χ	**0.229**	**0.216**	-0.112	-0.122	**-0.243**	**-0.300**	0.094	0.158
	P	0.011	0.012	0.886	0.892	0.993	1.000	0.179	0.045

Soil compaction and tree cover effects on grass production

Grass production, either in 2007 or 2008, was positively associated to organic matter (2-7 cm) and *Q. ilex* cover, but negatively with bulk density (2-7 cm), penetration resistance (9-14 cm) and *P. pinaster* cover (Table 4, Fig 4). There was not spatial co-variation between grass production and open sites. Mass water content was not associated to grass production.

Table 5. SADIE association tests between spatial patterns of compaction variables, cover type and annual grass production in two consecutive years. Χ (chi-p) is the index of overall association at *P* probability level. Associations are significant when *P*<0.025; dissociations are significant when *P*>0.975. Significant results are shown in bold text.

		Bulk density		Mass water content		Organic matter		Penetration resistance		*Pinus pinaster*	*Quercus ilex*	Open sites
Sampling depth		2- 7cm	9- 14 cm	2- 7cm	9- 14 cm	2- 7cm	9- 14 cm	2- 7cm	9- 14 cm			
Grass production 2007	χ	**-0.4268**	-0.1875	0.0770	-0.1170	**0.3773**	0.0900	-0.0576	**-0.2611**	**-0.4548**	**0.4346**	0.0601
	P	0.9999	0.9770	0.1975	0.8804	0.0001	0.2482	0.7396	0.9976	0.9999	0.0001	0.2683
Grass production 2008	χ	**-0.4808**	**-0.2731**	0.1501	-0.1149	**0.3718**	0.0947	-0.1020	**-0.3771**	**-0.4766**	**0.4648**	0.0040
	P	0.9999	0.9986	0.8488	0.8949	0.0001	0.1680	0.8576	0.9997	0.9999	0.0001	0.4844

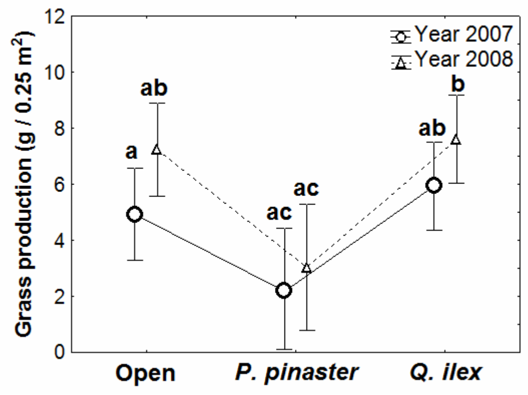


Figure 4. Grass production variation among years (2007 and 2008) depending of cover types. Significative differences are shown with different letters.

Tree cover-soil compaction-grass production into a spatial structural model

The presented model shows three levels: (1) tree cover, integrated by *Quercus ilex* (Qi) and *Pinus pinaster* (Pp); (2) soil compaction variables, integrated by bulk density (BD), soil organic matter (SOM), penetration resistance (PR) and mass water content (MWC); and (3) grass production (GP) of 2007 (model for both years is similar). A model for each depth will be commented separately:

Model a: 2-7 cm in depth

Goodness of fit to this model was higher than 0.9 for both indices (NFI=0.986, GFI=0.993) and χ^2 was non-significant (P=0.54 at 95% level). First level or tree cover presented a logical negative relation between presence of Qi and Pp. This fact is explained due to the site selection during pine plantation. With regard to second level or soil compaction variables, the first one only showed significative relations between Qi cover with bulk density BD (negative); Pp showed non-significant relation with BD and neither with SOM. Second level of soil compaction variables showed an important negative relation caused by SOM on BD. In turn, BD had a negative consequence on PR, but none effect on MWC. SOM did not show significative effect on PR and either on MWC. In turn, MWC had a negative consequence on PR. With regard to third level or grass production, the second only showed positive causal relation between SOM and GP. GP was negative affected by Pi cover (Fig 5a).

Model b: 9-14 cm in depth

Goodness of fit to this model was higher than 0.9 for both indices (NFI=0.958, GFI=0.982) and χ^2 was non-significant (P=0.10 at 95% level). First level or tree cover presented negative relation between presence of Qi and Pp. With regard to second level, the first one only showed significative relations between Qi cover with bulk density BD (negative) and SOM (positive); Pp showed non-significant relation with BD and neither with SOM. Second level of soil compaction variables showed a negative relation caused by SOM on BD. In turn, BD had a negative consequence on MWC, but none effect on PR (inverse situation than model a). SOM showed a positive significative effect only on MWC. In turn, MWC had a negative consequence on PR. With regard to third level or grass production, the second showed negative causal relation from PR and MWC over GP. GP was also negative affected by Pi cover at the same time that was affected positively by Qi cover (Fig 5b).

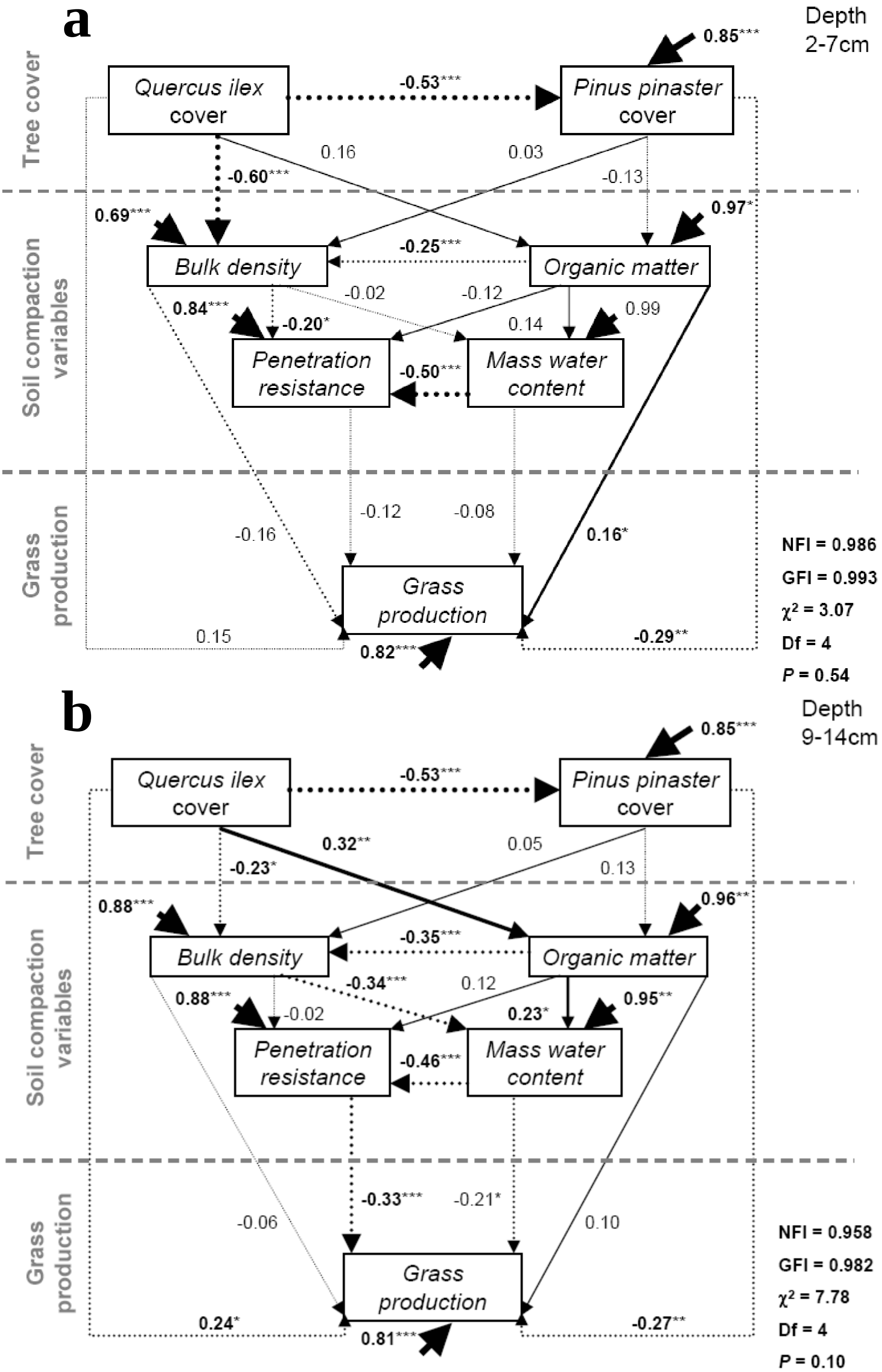


Figure 5. Structural equation model relating tree cover with soil compaction and grass production. A continuous line means a positive effect, a discontinuous line mean a negative effect. Arrow widths are proportional to path coefficient. Significant paths are indicate in bold text. See text for more details.

Discussion

Soil compaction in the space

Spatial heterogeneity in abiotic factors is an aspect which has been especially reported in the recent years due to its relevancy on plant dynamic ecology (Canham et al., 1994; Purves et al., 2007; Beckage and Clark, 2003; Jurena and Archer, 2003). Maestre et al. (2002, 2003) have found that soil heterogeneity on a small scale was closely related to plant distribution. Thus, soil physical properties manifest a pattern spatially explicit associated to the spatial pattern of plant survival. In our study, physical properties of soil (bulk density and penetration resistance), organic matter and humidity are aggregated along the space and in two sampling depths with a consistent pattern. These variables were also spatially associated: high organic matter was associated to low bulk density, and high penetration resistance was associated to low water content. Similar results were found by Quero et al. (2007) but depending of the type of habitat. Thus, in autochthons forest of *Pinus sylvestris*, penetration resistance was spatially dissociated with humidity, however this relation disappeared in shrubs areas and became positive (spatial association) in *P. sylvestris* plantations. Sojka et al. (2001) addressed the close relation between penetration resistance, soil water content and bulk density under field conditions, and how strength would increase with increasing bulk density and decreasing water content. With regard to organic matter as an important factor to ameliorate compaction, Franzluebbers and Stuedemann (2006) found the benefits carried by the organic matter-enriched surface soil are due to buffer-function against compactive forces. On the other hand, Logson and Karlen (2004) showed that higher amounts of organic carbon can result in a lower bulk density in some cases because organic carbon has a lower particle density than mineral particles.

Trees' footprint on physical properties of soil

Heterogeneity in soil physical properties was related to presence-absence of certain tree species. For instance, sites covered by *Quercus ilex* canopy exhibited higher organic matter content and lower bulk density (Fig. 3). On the other hand, open sites without tree cover had lower organic matter and higher bulk density values. These results are coherent with Gallardo (2003), who addressed the effect of *Q. Iex* canopy increasing soil organic matter content and how sites without tree cover present low N, P and K concentrations. In contrast, *Pinus pinaster* did not seem to modify soil physical properties, having similar values as open sites: higher bulk density and lower organic matter. Shukla et al. (2006) have found that tree cover modifies texture and soil organic matter content depending on the species. Thus, under *Quercus gabelli* canopy lower compaction was found in terms of bulk density than those sites covered by *Pinus edulis.* All these results may be underlying the important trees' role as an ecosystem engineer. For instance, *Quercus* genus seems to modify soil properties as a result of the high litter deposition which can be carrying out many different functions: increasing the nutrients availability, enhancing water retention and facilitation process for seedling establishment. However, *Pinus* genus did not change soil compaction because of its low litter deposition linked to its low decomposition rate. In addition compaction reduces mineralisation rate due to the low aeration which would explain the high bulk density linked to its low organic matter (Breland and Hansen, 1996). Thus, soil compaction under *Pinus* canopy could enhance water runoff and fine soil particles loss.

Grass production in a mosaic of tree cover and soil compaction

Grass production was linked to soil physical properties and tree cover. Thus, productivity was higher in those sites covered by *Q. ilex* instead of *P. pinaster*. Many works have shown the pines role decreasing grass productivity because of its allelopathic exudates. At the same time, *Quercus* effects have been reported as beneficial due to its role of increasing organic matter. Despite this positive effect we would expect a trade-off with other abiotic factors such as light. Thus, although there was not an associate pattern between open sites and grass productivity, in absolute values the biomass production was almost the same in open and below *Quercus*. On the other hand, higher production values were found linked to low bulk density values, high organic matter content, and low penetration resistance. Godefroid and Koedam (2004) described grass species response to compaction under field conditions as a wide spectrum effect from positive, null or negative. In general, there was a negative effect of soil compaction over herbaceous performance. The higher productivity associated to high organic matter content may be caused by a feedback loop. On the one hand, sites with higher organic matter have higher nitrogen concentration, which is one of the limiting factors for grass production. On the other hand, sites with high primary production tend to have higher litter depositions rates. Therefore, the organic matter increment would be the result of litter decomposition, allowing a new nutrients reserve for the next annual species. All these association patterns between tree cover, soil variables and herbaceous productivity are consistent in time, being practically the same for 2007 and 2008. Therefore, there seems to exist a strong structure linking plant cover and soil throughout space and time.

Conclusion

Soil variables associated to compaction (bulk density, penetration resistance, water content and organic matter) exhibit an aggregate spatial pattern. This pattern was closely related to trees canopy, but the effects depend on the species. *Quercus ilex* has an important effect on soil surface properties (2-7 cm) as the higher organic matter produced a reduction in bulk density. *Pinus pinaster* is not able to modify soil physical properties, having similar properties as open sites: high bulk density and low organic matter. These relationships between soil compaction and tree canopy have implications on grass productivity in spatial and temporal terms.

Acknowlegments

Very special thanks to Raquel Casado and Victoria González for setting up the whole plot. Thanks to Elena Suárez, Manolo Olmo and Kris Calero for the field work assistance. To Enrique Ramírez for the topography analysis and to Alfonso and Ignacio García-Ferrer for letting us utilize the total station. To José Luis

Quero for instructing us in the SADIE technique. To Cristina Gómez, David Gómez and José Manuel Recio for assisting us during the soil analysis. To Txema Iriondo and Adrián Escudero for teaching us all that we know about SEM. This work was financed by DINAMED project (CGL2005-05830-C03-02/BOS) and INTERBOS project (CGL2008-04503-CO3-02) through the Ministry of Science and Innovation of Spain and co-financed with FEDER founds (European Union).

Chapter 3

Moderate soil compaction: implications on growth and architecture in seedlings of 17 woody plant species

Published in:
Alameda, D., Villar, R. 2009. Moderate soil compaction: implications on growth and architecture in seedlings of 17 woody plant species. Soil & Tillage Research 103, 325-331.

Moderate soil compaction: implications on growth and architecture in seedlings of 17 woody plant species

Abstract

Generally, soil compaction is a stress factor affecting negatively the plant growth, but its effects vary between species and with the soil compaction range. The objective of this study is to know the different growth responses of 17 woody species subjected to moderate soil compaction, because most of the studies about this subject compare the effects in treatments with a wide and discrete compaction range. We explore the effects of moderate soil compaction on a continuous scale (0.1- 1.0 MPa) on seedling growth. Seedlings of 17 woody species (deciduous and evergreens) mainly from Mediterranean ecosystems were grown in near optimal conditions (light, temperature and water) in a greenhouse with a sandy substrate.

In general, there was a great variability of the responses depending on the species and the studied variable. About 53 % of the species showed a higher total biomass with a moderate increase in soil compaction possibly being due to a greater root-soil contact. In the same way, 41 % of species increase the relative growth rate and 35 % the total area. Nevertheless, in spite of these positive effects on growth, for some species (23%) there was a decrease in the root proportion with soil resistance, as result of soil strength.

These effects of moderate soil compaction could sum up in two general responses of woody plants: growth increment and architecture distortion. This might be relevant as a lower root investment may be a disadvantage under drought conditions. Finally, a simple conceptual model is proposed to understand the general effects of soil compaction on growth and biomass allocation.

Keywords: biomass allocation, Mediterranean ecosystem, penetration resistance, relative growth rate.

Resumen

Generalmente, la compactación del suelo es entendida como un factor de estrés que afecta negativamente el crecimiento vegetal, sin embargo, sus efectos varían según la especie y el rango de compactación estudiado. El objetivo de este trabajo es conocer las diversas respuestas del crecimiento de 17 especies leñosas a la compactación moderada del suelo, ya que la mayor parte de los estudios realizados emplean tratamientos con un rango amplio y discreto de resistencia a la penetración. Estudiamos los efectos de la compactación moderada de un sustrato arenoso en una escala continua de resistencia (0.1 - 1.0 MPa) sobre el crecimiento de plántulas crecidas en condiciones óptimas de invernadero.

Hubo una gran variabilidad de las respuestas dependiendo de la especie y de la variable estudiada. El 53% de las especies mostraron un incremento de la biomasa total con el aumento de la compactación, posiblemente debido a un mayor contacto raíz-suelo. Del mismo modo, el 41% de las especies vieron incrementada su tasa de crecimiento relativo y un 35% su área foliar total. Sin embargo, a pesar de estos efectos positivos sobre el crecimiento, para algunas especies (23%) hubo una disminución de la proporción de raíz con el aumento de la resistencia a la penetración.

Los efectos de la compactación moderada del suelo en plantas leñosas pueden resumirse en dos respuestas generales: incremento del crecimiento y distorsión de la arquitectura. Esto puede ser relevante bajo condiciones de sequía, donde una inversión menor en raíz puede ser una desventaja. Finalmente, se propone un modelo conceptual para entender los efectos generales de la compactación del suelo sobre crecimiento y distribución de biomasa.

Palabras clave: *distribución de biomasa, ecosistemas mediterráneos, resistencia a la penetración, tasas de crecimiento relativo.*

Introduction

Soil compaction is a well established corpus of investigation generating over 3000 references in the last quarter century (ISI Web of Knowledge, February 2008). Many aspects of the effects of soil compaction have been studied. Kirby (2007) in his note discussed what we know and what not about soil compaction, concentrating mainly on the effects on crops and farms and on the ways to ameliorate these effects.

Although most of the problem of soil compaction has a human origin, soils of natural areas also show a different degree of natural compaction (Perez-Ramos, 2007; Quero et al., 2008; Gómez-Aparicio et al., 2008). A high soil compaction may have a strong effect on the first phase of establishment of wild plants (Perez-Ramos, 2007; Gómez-Aparicio et al., 2008; Pérez-Ramos et al., 2008). Not many studies about soil compaction deals with these aspects and not much information is known about the response of wild woody species to soil compaction. In fact, Kirby (2007) appealed to re-direct compaction research on broader issues as for example natural resource management and biodiversity.

In general, at a whole plant level, it is assumed that soil compaction affects negatively root investment, due to the resistance of the substrate to be penetrated (Day and Bassuk, 1994; Kozlowski, 1999; Bassett et al., 2005). Verdu and Garcia-Fayos (1996) found that root penetration of Pistacia lentiscus was negatively associated with soil compaction. Similarly, Bejarano et al. (2005) found in Quercus pyrenaica a decrease in root length with soil compaction. This may determine that in situations of water deficit, plants with a lower root mass proportion or shorter roots could suffer a higher water deficit which might seriously limit seedling survival. In fact, Lloret et al. (1999) stressed the importance of a greater root mass proportion (RMR, root mass ratio) in Mediterranean environments because species with a greater RMR displayed a greater survival after the period of summer drought, due to the ability to explore a greater soil surface and/or deeper soils.

Moreover of the effects on root investment, plant growth is also affected by soil compaction. In general, plant growth has been found to show a clear negative effect. Basset et al. (2005) found in *Cordyline australis* that stem and root growth was negatively affected by soil compaction. As stated before, most of the studies on the effect of soil compaction on plants are done mainly on herbaceous species, and a minor proportion on wild woody species. Therefore, in the present study, we analysed the effects of soil compaction on the absolute growth and relative growth rate of 17 woody seedling species. Up to now, no work exists that studies the effect of soil compaction on relative growth rate (RGR) and the underling growth components.

The relative growth rate (RGR_M), which is defined as the increase of biomass per unit of biomass and time may also be affected by soil compaction. A high RGR_M could be advantageous for a plant as it allows a greater biomass in less time (Van Andel and Biere, 1989), which would allow it to obtain more resources (light, water and nutrients) and confer a greater competitive capacity (Grime, 1977). Variation on RGRM between different species has been found to be mainly related to a morphologic variable: the leaf area ratio (LAR; the amount of leaf area divided by the total biomass) (Poorter and Remkes, 1990). Variation in LAR is due to two components: the specific leaf area (SLA; the ratio of leaf area to leaf mass); and the leaf mass ratio (LMR, the proportion of leaf mass), being LAR = SLA x LMR. On the other hand, other morphological variables can also influence RGRM like the proportion of biomass allocated to stem (SMR) and root (RMR), which are related to LMR.

In this comparative study, seedlings of 17 woody species, including deciduous and evergreens species, were grown for testing the influence of leaf habit on soil compaction response. These two functional groups have contrasting traits, as deciduous species usually present high photosynthetic and respiration rates, high leaf nitrogen concentration, and specific leaf area (Reich et al., 1992; Aerts 1995; Villar et al., 1995; Reich et al., 1997; Wright et al., 2004; Quero et al., 2006). Consequently, deciduous species usually grow faster than evergreens ones (Reich et al., 1992; Cornelissen et al., 1996; Antunez et al., 2001; Ruiz-Robleto and Villar, 2005). Also important differences in biomass allocation exist, as deciduous tree species invest higher proportion of biomass to roots (high RMR) than evergreens (Villar et al., 2004). As soil compaction might have an effect on root investment, the two functional groups may respond differently.

Seedlings were grown in a greenhouse with a sandy soil texture and constant water content. Therefore, the effect of a different texture or different soil moisture on soil compaction was avoided, as it has been found by Smith et al. (2001) and Souch et al. (2004). Another novel approach is that we have used a continuous range to investigate the effects of soil compaction. Most studies have analysed the effect of soil compaction using few levels of soil compaction. Only few studies have analysed the effects in a continuum soil compaction (Basset et al., 2005). In a general ecological framework, Cottingham et al. (2005) advised the use of regressions as opposed to ANOVA for understanding the effects of different factors, because regression has a greater predictive power and the possibility to obtain predictors which can be used in ecological models.

In summary, the aims of this study are: 1) investigate the effect of moderate soil compaction on growth and other growth variables in an ample number of woody species; 2) compare the effect of soil compaction on evergreens and deciduous; and finally 3) to elaborate a general conceptual model of the effect of soil compaction on growth and biomass allocation.

Material and methods

Experimental design

The experiment was developed in a greenhouse with temperature control at the Campus of University of Córdoba (Spain) between January to July 2006. The mean ± SD of the photosynthetic active radiation measured (with EMS7, canopy transmission meter, PP-system, UK) during a clear day (May 8th, 2006) was 446 ± 89 µmol photon m-2 s-1, and the total daily mean radiation throughout the experiment was 40.6 mol m-2 day-1. Water was provided everyday by a dripping irrigation system. At 3 months of growth 1 g of slow-release fertilizer (Plantacote Pluss, NPK 14:9:15, Aglukon, Valencia, Spain) was added to avoid nutrient deficits.

The substrate was a mixture of river sand, black peat and lime in a proportion 9:6:5, having a sandy soil texture (88% sand, 8% silt and 4% clay). The soil had very similar characteristics to those found in Q. ilex "dehesas" (savannah-like ecosystems) of the province of Cordoba, with a 77 % of sand proportion and 1.29 g cm-3 of bulk density (Fernandez-Rebollo et al., 2004). The pots were made of PVC (4.33 L) which were 50 cm in height and 10.5 cm in diameter, to avoid limitation of space for root growth. The base of the pots (a square of 12 x 12 cm) was perforated with 5 orifices of 2 mm diameter to allow a good drainage.

Eight treatments of soil compaction were made in order to obtain a continuous soil compaction range using bulk density increment as reference. The first level (null compaction) was made filling the whole volume of the pot with soil without compacting it. The following compaction levels (from 2 to 8) were made increasing the pot weight by 265 g in average for each level by adding more substrate. Therefore, pots of level 8 weighted about 1900 g more than level 1. The substrate was manually compacted with a metallic rod (10 cm diameter). Initially, all pots were filled up with substrate about 49 cm of height. However, in the lower compaction treatments the substrate height was reduced after few days of watering and therefore its soil volume decrease in these treatments. Soil compaction levels were related to soil penetration resistance and bulk density increment (Table 1).

The total number of pots was 408, distributed in 8 levels of soil compaction with 3 replicates by each soil compaction level for each of the 17 species studied.

Table 1. Mean values (± SD) of soil characteristics of compaction treatments. VWC is the volumetric water content measured with a TDR. MWC is the mass water content.

Treatment	Mean penetration resistance (MPa)	Maximum penetration resistance (MPa)	Bulk density (g cm^{-3})	VWC (%)	MWC (%)
1	0.13 ± 0.08	0.42 ± 0.54	1.12 ± 0.12	21.57 ± 7.19	18.96 ± 5.64
2	0.12 ± 0.06	0.44 ± 0.26	1.09 ± 0.08	20.86 ± 5.41	18.99 ± 5.03
3	0.16 ± 0.08	0.51 ± 0.40	1.10 ± 0.08	23.22 ± 6.33	20.09 ± 5.24
4	0.16 ± 0.06	0.47 ± 0.24	1.19 ± 0.07	21.55 ± 5.29	17.65 ± 3.55
5	0.17 ± 0.06	0.50 ± 0.23	1.19 ± 0.10	23.11 ± 5.68	18.30 ± 6.69
6	0.25 ± 0.07	0.57 ± 0.23	1.23 ± 0.09	24.59 ± 6.00	18.3 ± 4.47
7	0.35 ± 0.16	0.75 ± 0.45	1.26 ± 0.07	24.23 ± 7.21	17.17 ± 4.82
8	0.58 ± 0.18	1.16 ± 0.51	1.28 ± 0.10	28.61 ± 7.05	18.7 ± 5.32

Growth

Woody species were chosen from families representative of the Mediterranean forest with deciduous and evergreen species. Most species were autochthonous, but some were naturalised (*Ceratonia siliqua*), ornamentals (*Cercis siliquastrum*) or invaders (*Ailanthus altissima*). In general, 40-50 seedlings of each species were selected when seedlings had a pair of expanded leaves or had a root length of 3 - 4 cm (for *Quercus* species). For each species, individuals with a similar size were selected to avoid a high variability between individuals and have a more precise RGR_M estimate (Poorter and Garnier, 1996). An initial harvest was done to have an initial biomass estimate for each species. For that, between 10-15 seedlings of each species were fresh weighed and dried at 70 °C for at least 2 days, then the dry mass was weighed. As *Quercus* seedlings had only a small root, we measured fresh and dry root mass and root length, to build a predictor for root biomass. With all this data we have an estimate of the initial mass and the dry matter content (ratio of dry mass to fresh mass) for each species. The remaining plants were weighed individually (obtaining the plant fresh mass) and then planted in the pots. Each seedling was planted making a small hole of a few centimetres in the substrate to place the root. The mean initial root length for all the 17 species was 5.3 ± 3.4 cm.

Approximately, after 137 days of growth plants were harvested. Previously for each plant three measures of soil penetration resistance were taken each 0.5 cm using a penetrometer (developed by the Department of Agroforesty Engineering, University of Cordoba) with a cone of 12.6 mm diameter and 30° of angle. For each pot, we use the average value of the three measurements of soil penetration resistance for the whole soil profile (approximately 45 cm) as a representative value of soil compaction. Additionally, three measures of water volumetric percentage in the upper and in the lower 20 cm of the substrate were taken with a TDR (FieldScout TDR 100, Spectrum Inc. Technologies). The mean value of water volumetric percentage of the soil was 18 ± 5% (for the upper 20 cm) and 30 ± 8% (for the down 20 cm). We did not find any significant correlation between soil water volumetric percentage and plant biomass for any of the studied species (data not shown). The harvest of each plant was made by extracting carefully the plant from the pot and roots were washed in a container with water. Roots were gently dried out, maximum root length and plant fresh mass were measured. Then fresh leaves, stems and roots were weighed. Fresh leaves were stored in plastic bags with humidified paper and placed in a cool box. A sub sample of fresh leaves was scanned and the area measured with an image analyzer (Image Pro, v4.5; Average Cybernetic, Inc., Silver Spring, Md, USA). After these measurements, leaves, stems and roots were dried at 70 °C for at least two days to obtain the dry biomass.

Analysis of growth

The relative growth rate was calculated following the classical approach (Hunt 1990) as: $(LnM_2 – LnM_1)/ (t_2 – t_1)$, being M_2 and M_1 the final and initial dry mass of the seedling respectively and t_2 - t_1 the growth period. Final dry mass for each plant was obtained at the harvest after the period of growth. Initial dry mass for each plant was obtained as: initial fresh mass × dry matter content. The initial fresh mass for each plant was obtained weighing each seedling at the time of planting. The dry matter content (dry mass/ fresh mass) was obtained from a sub sample of plants (10-15 seedlings per species) at the beginning of the experiment (see above). For *Quercus* species, most of the initial fresh mass was due to the seed and only a minor amount to the root, therefore we calculate the biomass of the root using a predictor for each species. For that, a regression between fresh root mass and root length was made, giving acceptable predictions (R^2 average of 0.62 ± 0.15 for the 6 species of *Quercus*). Then, the initial fresh mass was transformed to initial dry mass using the dry matter content of the roots.

The growth variables were calculated following Hunt (1990). Specifically, the proportion of leaves (LMR, leaf mass ratio), stems (SMR, stem mass ratio) and roots (RMR, root mass ratio) were calculated as the ratio of dry biomass of leaves, stems and roots respectively and total dry biomass. Specific leaf area (SLA) was calculated as the ratio of leaf area and leaf dry mass. The leaf area ratio (LAR, the total leaf area per unit of total plant dry mass) was calculated as the product of SLA and the proportion of leaves (LMR). In May and July two stem height measurements were taken for each plant. RGR height (RGR_H) was calculated as $(LnH_2 - LnH_1)/(t_2 - t_1)$, being H_2 and H_1 the stem height at the different times (t_2 and t_1).

Statistical analysis

In order to see the influence of soil compaction on the different variables measured, multiple regressions were performed for each species. Because the soil compaction treatments showed a continuous range in soil penetration resistance (from 0.1 to 1 MPa), the regressions were the most appropriate test to determine the influence of soil compaction on growth variables. Multiple regression models used as independent variables: soil penetration resistance (SPR) and SPR^2. Therefore, three types of results could be obtained: non significant effect, a linear regression or a polynomic regression. The polynomic regression could be of three forms ($x + x^2$; $x - x^2$ or x^2). We use the procedure of multiple stepwise regression for each dependent variable and species and those models with a higher R were chosen.

To evaluate soil compaction influence on species with a different leaf habit (evergreens versus deciduous) a covariance analysis was carried out (ANCOVA). For that, we chose leaf habit as the covariate, the growth variable as the dependent variable and soil penetration resistance as the continuous predictor. All statistical tests were made with Statistica 7.1 (StatSoft, Inc.).

Results

Biomass, relative growth rate and total leaf area

In general, we obtained three types of responses of the effects of moderate soil compaction on growth variables: non effect, linear positive effect and a polynomic response ($x + x^2$) effect with parabolic dynamic (Table 2, Fig. 1). For brevity only six species have been choosen to show the different effects of a moderate soil compaction (Fig. 1).

Table 2. Species studied and correlation coefficients for different variables and soil compaction.

Family	Species	Leaf habit	N	Growth variables				Architecture variables			
				Total dry biomass	RGR$_M$	RGR$_H$	Total area	R/S	RMR	SLA	LAR
Fagaceae	***Quercus ilex*** subsp. ***ballota*** (Desf.) Samp**.**	E	32	--	--	0.41 *	--	--	--	--	--
	Quercus coccifera L.	E	32	--	--	0.65 ***	--	-0.38 *	-0.40 *	--	--
	Quercus suber L.	E	23	0.54 * $_{(2)}$	0.54 * $_{(2)}$	--	--	0.48 $^{a}_{(3)}$	0.51 * $_{(3)}$	--	--
	Quercus faginea Lam.	D	31	--	--	0.77 ***	0.52 **	-0.41 *	0.50 * $_{(3)}$	--	0.43 *
	Quercus canariensis Willd.	D	30	--	--	--	--	0.40 $^{a}_{(3)}$	--	--	--
	Quercus pyrenaica Willd.	D	24	--	--	--	--	--	--	--	--
Simaroubaceae	***Ailanthus altissima*** (Mill). Swingle	D	22	0.45 *	--	--	0.48 *	--	--	0.53 * $_{(4)}$	--
Rhamnaceae	***Rhamnus alaternus*** L.	E	22	0.49 *	0.48 *	--	0.58 * $_{(2)}$	0.54 * $_{(3)}$	0.53 * $_{(3)}$	--	--
	Frangula alnus Mill.	D	24	0.42 *	0.46 *	--	0.35 ^{a}	--	--	--	--
Pinaceae	***Pinus sylvestris*** var. ***nevadensis*** H. Christ.	E	24	--	--	--	--	--	--	-0.57 **	--
Anacardiaceae	***Pistacia lentiscus*** L.	E	24	0.45 $^{a}_{(2)}$	0.54 * $_{(2)}$	--	0.45a $_{(2)}$	--	--	--	--
	Pistacia terebinthus L.	D	24	0.56 **	0.44 *	--	0.50 *	--	--	0.56 * $_{(3)}$	-0.41 *
Rosaceae	***Prunus lusitanica*** L.	E	24	--	--	0.37 $^{a}_{(4)}$	--	--	--	--	--
Caesalpinaceae	***Ceratonia siliqua*** L.	E	24	0.61 ** $_{(2)}$	--	0.56 *	--	--	--	0.56 * $_{(3)}$	0.57 * $_{(3)}$
	Cercis siliquastrum L.	D	23	0.46 *	--	--	--	--	--	-0.42 *	--
Oleaceae	***Olea europaea*** var. ***sylvestris*** (Mill.) Lehr	E	24	0.57 * $_{(2)}$	0.67 ** $_{(2)}$	-0.36 ^{a}	0.52 * $_{(2)}$	--	--	0.44 $^{a}_{(3)}$	--
	Fraxinus angustifolia Vahl.	D	22	0.70 ** $_{(2)}$	0.64 ** $_{(2)}$	--	0.68 **	--	--	--	--
	% Significative effects			53	41	23	35	17	23	30	17
	Mean R^2 (x 100)			29	34	37	30	20	24	28	23

Leaf habit: evergreen (E) and deciduous (D). N is the number of replicates for each species. Significant correlations are displayed: a $0.05 < P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. RGRM: relative growth rate in a biomass basis; RGRH: relative growth rate in a stem height basis; Total area: plant total area; R/S: root-shoot mass ratio; RMR: root mass ratio; SLA: specific leaf area; LAR: leaf area ratio. Nomenclature follows Lopez-Lillo and Sanchez-Lorenzo (2001). Subscripts indicate the type of polynomial regression: (2) $x - x^2$; (3) $- x + x^2$; (4) $-x^2$. Those numbers with no subscripts mean a lineal regression. % Significative effects is the number of species with a statistically significant effect of soil compaction divided by the total number of species (17).

Ten out of 17 species (53 %) showed a significant increase of total biomass with soil penetration resistance (Table 2). For five species the relationship had a bell shape (Fig. 1), having an increase in total biomass of up to about 0.4 MPa of soil resistance, afterwards showing a decrease in total biomass.

Seven out of 17 species (41 %) also showed a significant increase of relative growth rate on a biomass basis (RGR_M) with soil penetration resistance (Table 2). Similarly, as for biomass, for some species the relationship had a bell shape (Table 2). In relation to the relative growth rate on a height basis (RGR_H), we also found for four species a significant increase with soil compaction (Table 2). However, the results on RGR_H were very different of those to RGR_M (on biomass basis), because for both variables (RGR_H and RGR_M) none of the species with a significant effect of soil compaction were the same (Table 2). For many species RGR_M was not correlated ($P > 0.05$) with RGR_H, only five out of 17 species (Q. canariensis, C. siliquastrum, P. sylvestris, P. lusitanica and P. lentiscus) showed a positive and significant correlation between RGR_M and RGR_H, but these showed a low percentage of variation explained (mean R^2 of 0.24).

Total leaf area showed a similar trend to that found for total biomass, for seven species (35 %) there was a significant increase with soil compaction. Four out seven species showed a linear increase with soil compaction and three species had a bell shape (Table 2 and Fig. 1).

The percentage of variance of these growth variables explained by the effect of soil compaction was between 29 to 37 % (R2 x 100; Table 2).

Biomass allocation and SLA

For 17 % of species Root/Shoot ratio (R/S) had a negative relationship with soil compaction, that is, a decrease of root biomass respect to shoot biomass. Similarly, four out of 17 species (23 %) showed a decrease of root biomass ratio (RMR) with soil resistance (Table 2 and Fig. 1). In both variables, apart from a linear negative response there was a polynomic response ($-x + x^2$), showing a decrease at low soil resistance and reaching an asymptote on high values of penetration resistance (Fig. 1, see *Q. suber* and *R. alaternus*).

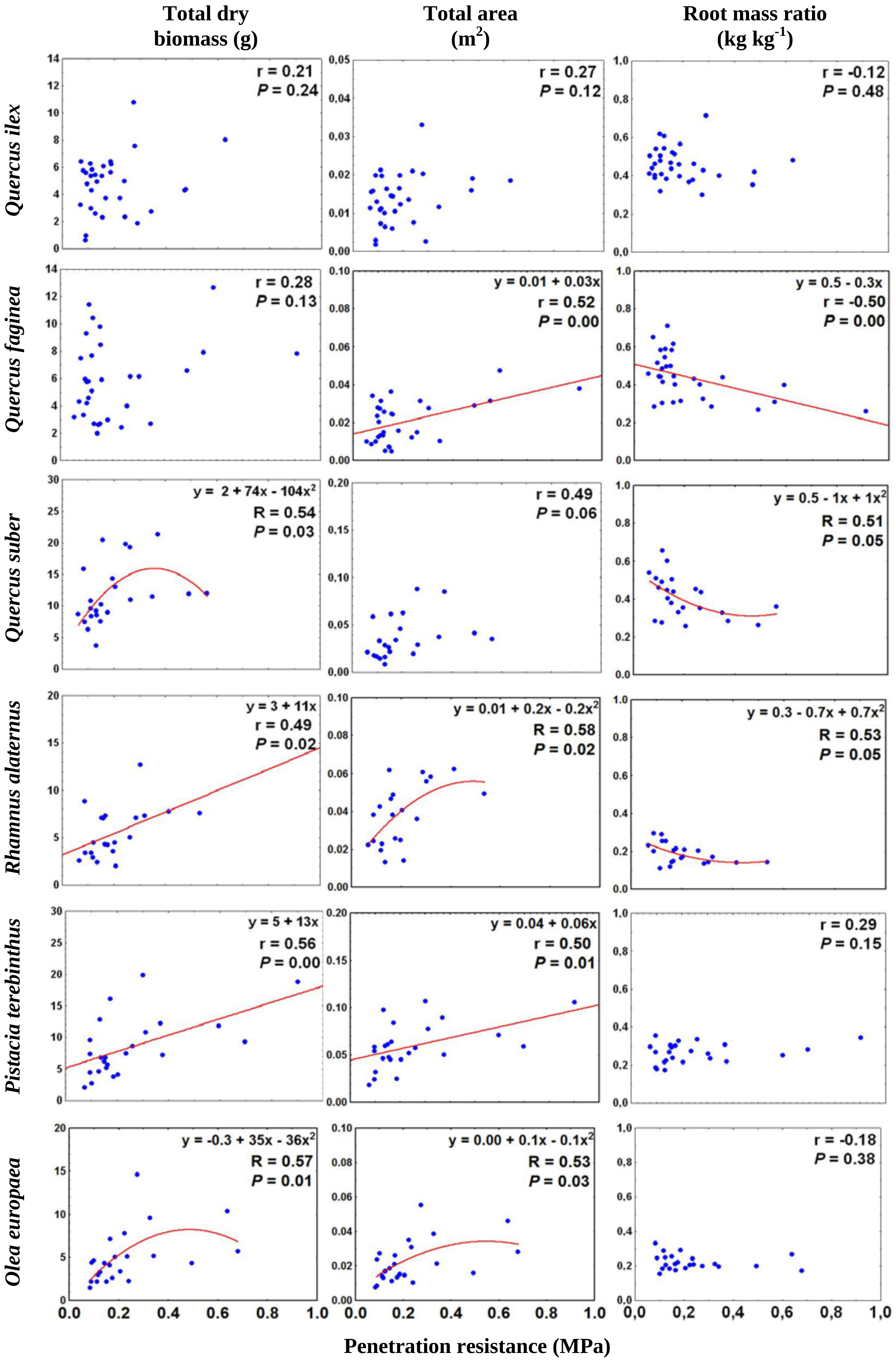


Figure 1. Relationships between soil penetration resistance and some growth variables [total biomass, total leaf area and root mass ratio (RMR)] in 6 woody species. Species with a different response were selected. For each graph, the correlation coefficient (r) or in cases of a polynomial regression the multiple R and P are indicated.

Five out of 17 species (30 %) showed a significant effect of soil resistance to SLA (Table 2), having a negative linear response or a polynomic response of type -x + x^2. Also, three out of 17 species (17 %) showed a significant effect of soil resistance to LAR but some species showed a linear decrease (*P. terebinthus*) and other species a positive relationship (*Q. faginea*) (Table 2).

Leaf habit

In general, the two functional groups respond differently to soil compaction for many of the variables measured. The ANCOVA showed a significant Leaf habit x Soil Resistance interaction (data not shown) for leaf, root and total biomass, total area and Shoot/Root ratio. Deciduous species showed a higher and positive effect of soil resistance on leaf, root and total biomass, total area and Shoot/Root ratio than evergreen species.

Discussion

Soil compaction is one of the stress factors that can affect the development of plants (Kozlowski, 1999), nevertheless, little has been studied from an ecological point of view. Most of the studies have been made with herbaceous or woody species of agricultural, forest or ornamental interest. Very few studies have been focused on wild woody species (e.g. Mósena and Dillenburg, 2004; Basset et al. 2005, Perez-Ramos, 2007). The present work has several novel points: the study of the effect of soil compaction on an ample number of wild woody species (17), the comparison of two different plant strategies (deciduous and evergreens) and the study of the effects of moderate soil compaction on biomass, relative growth rates and their growth components. Most of the studies on biomass and relative growth rate have been focused on the effects of other conditions and resources (light, water, nutrients, temperature) (see for example Lambers et al., 1990 and Lambers et al., 1998), but very few on the effects of soil compaction.

In the present work, the seedling phase has been studied since it can be the most sensitive stage, as found by Souch et al. (2004), since the young roots, of slight thickness, are those that have to colonize the soil and have to overcome the soil resistance. On the other hand, the sandy texture used, slightly compactable, has allowed us to create moderate levels of compaction (from 0.1 to 1.0 MPa), but very similar to the levels found in soils from "dehesas" (savannah-like ecosystems) (Fernandez-Rebollo et al., 2004) in the Iberian Peninsula. Also, other studies (Godefroid and Koedam, 2004; Souch et al., 2004; Basset et al., 2005) have maximum values of soil compaction around 1.2 - 1.4 MPa.

In general, the results of our study show that moderate soil compaction affects differently to woody species. Different responses have been found, from null effect, positive or negative linear effects or a bell shape response on growth and growth components. Similarly, Godefroid and Koedam (2004), found a very diverse influence of soil compaction on the plant cover of 107 herbaceous species. In that study, most of the species showed a bell shape response with respect to soil compaction, with a maximum plant cover between 0.2 to 0.6 MPa, decreasing it at higher soil compaction values.

In our study, 53 % of the species showed a significant increase of total biomass with soil resistance. Similarly, RGR_M for some species was also increased with a moderate increase in soil resistance. In general, the results found in the literature have been a negative effect on total biomass and growth. However, most of the studies have higher soil compaction ranges and they use a discrete approach. Few studies have found similar results to ours. For example, Mósena and Dillenburg (2004) found also for *Araucaria angustifolia* an increase in biomass with higher soil compaction. These results would be coherent with some agro-forestry practices directed to increase plant growth and to facilitate the contact of root with soil, consisting of the induction of a moderate compaction at sowing or planting. In fact, some studies (Arvidsson, 1999; Gomez et al., 2002b) have found that a moderate soil compaction can determine a greater contact between the root and the substrate and allow for a greater water and nutrient absorption.

However, in spite of the positive effect of a moderate soil resistance on the total biomass for some species, the architecture of the plants was negatively affected, concretely the root mass ratio (RMR). A general result found in the literature is a negative effect of the soil resistance on the root, measured as a decrease in root penetration (Verdu and Garcia-Fayos, 1996), root growth (Conlin and van den Driessche, 1996; Bassett et al., 2005) or root investment (Kozlowski, 1999). This can determine that in situations of water deficit, plants with a lower root development may suffer drought more severely, and therefore it could seriously limit seedling survival (Lloret et al., 1999). Studies to test this hypothesis are lacking.

However, although the effect is statistically significant for few species, the percentage of variation of the root growth explained by soil compaction is usually very low. For example, Bassett et al. (2005) using regressions, found that soil resistance explained a very small percentage of the variation (between 16 to 22%) of the root growth. This agrees with our results since in the cases in which there is a significant effect, the explanatory percentage of soil compaction on plant traits (R^2 x 100) is between 20 to 37 % (Table 2). It is expected that higher soil compaction levels (> 1.5 MPa) may have a higher effect on plants.

In relation to the differences between deciduous and evergreens species, our hypothesis was that soil compaction could have a different effect in these two functional groups, since in general deciduous invest more biomass to roots (higher RMR and largest root pattern) (Antunez et al., 2001; Villar et al., 2004; Ruiz-Robleto and Villar, 2005). We have found that evergreens responded less to the soil compaction than deciduous, which could be interpreted that evergreens have therefore a smaller level of plasticity. Similarly, results of other studies (Valladares et al., 2000) suggest that evergreens species are less plastic to changes in conditions and resources.

As in other studies about soil compaction, the obtained results are subject to the experimental conditions, concretely to a sandy texture, optimal conditions of water and moderate levels of compaction. It is well known that soil penetration resistance is very dependent on the soil texture and water content (Smith et al., 2001; Souch et al., 2004). Gomez et al. (2002a) found that the effect of soil compaction on the growth of saplings of Pinus ponderosa could be negative, insignificant or positive, depending on the texture or water content of the soil. Therefore, it is very difficult to extrapolate the results to other soil conditions. Overall, the fact that all the plants were in the same conditions is an important advantage, since it allowed the study of the effect of soil compaction, without other interacting variables, such as soil texture or soil water content.

A conceptual model

The general trends in our results, allow us to propose a general conceptual model where most of the tendencies found can be synthesized. Two fundamental aspects are of interest: a) the biomass or growth and b) the plant architecture.

On one hand, we have found that growth measured like total dry biomass, displays two types of response (Fig. 2 A). Species type I are those in which we found an increase of biomass (or another variable of growth) with an increase of soil compaction until moderate levels. At greater values of soil compaction some species started to decrease growth (as has been found in our study and by other authors). Arvidsson (1999) found a bell response in *Hordeum vulgare*, with low yield in the lowest and highest soil compaction. The other type of response are the species type II, which showed no effect on growth (at the measured levels of compaction), but that would show a reduction of growth at greater levels of compaction, as has been suggested in the literature.

In relation to the plant architecture (e.g. the root proportion), we have found two main types of response (Fig. 2 B). Species type A which showed a reduction of RMR with an increase in soil compaction, and at much greater levels of compaction they would reach a minimum RMR. Species type B where those that did not show any effect of moderate soil compaction on RMR, but they probably change RMR at higher levels of soil compaction. Within this general scheme, we could find species showing all the possible combinations of these responses. For example, *Rhamnus alaternus* follows type I-A, *Pistacea terebinthus* follows I-B, *Quercus faginea* II-A and *Quercus ilex* follows II-B.

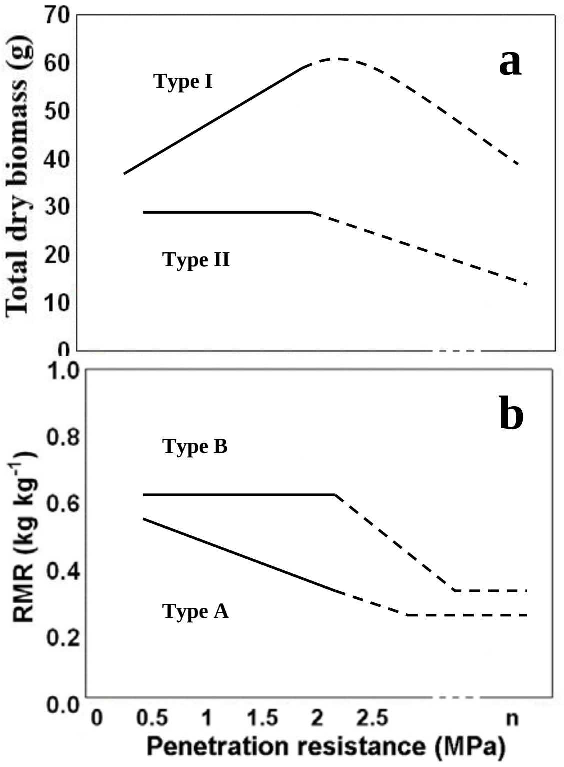


Figure 2. Conceptual model summarising the effects of soil penetration resistance on total dry biomass (a) and root mass ratio (RMR; b). Solid lines indicate the response found in this study under moderate levels of soil compaction (< 1 MPa) and slash lines indicate the possible response under higher levels of soil compaction (found in literature).

Conclusion

In summary, in this work we have found a great variability of responses to soil compaction which is dependent on the variable and the species studied. The effects of a moderate soil compaction on the studied variables varies from positive or null effects on growth and biomass but null or negative on plant architecture, concretely a decrease of the root proportion, that it could have great consequences for survival. In general, it is proposed that soil compaction would have a bell effect for growth, where we would have values of non effect, a central range of positive effect (on the growth) and a negative effect for high values of soil compaction.

Acknowledgements

Very especially we thank all those people who disinterestedly have participated in the development of some stages of the experiment: Nani, Cristina, Victor, Emilio, Vico, Ana, Ocaña, Álex and Kike; or who have simply given support: Carmen, Laura, María, David, and Joaquín; especially to Eli for the moral and physical support. We thank Juan Agüera for lending us the penetrometer and the advice on its use; Antonio Sánchez ("Viveros de San Jerónimo", Junta de Andalucía) provided Olea, Pistacia and Ceratonia seedlings. Seeds of several species were kindly supplied by many friends: Nacho Pérez and Teodoro Marañón (Q. canariensis), José Luis Quero (P. sylvestris) and Fernando Pulido (P. lusitanica). Fraxinus seeds were provided by Banco de Germoplasma (Córdoba). Lourens Poorter and two anonymous referees give interesting comments that improve the paper. Part of this work was financed with the project DINAMED "Dynamic of the Mediterranean forest in a global change scenario: ecophysiological basis (CGL2005-05830-C03-02/BOS, Ministry of Education and Science, 2005-2008).

Chapter 4

Compactación moderada del suelo: implicaciones en la respuesta a la sequía de 12 especies leñosas y el efecto de la lluvia estival

Partially published in:
Alameda, D., Villar, R. 2009. Estrategias de respuesta a la sequía en plántulas de 17 especies leñosas y el efecto de la lluvia estival. En: 5º Congreso Forestal Español. Montes y sociedad: Saber qué hacer. ISBN 978-84-936854-6-1. Ed: Sociedad Española de Ciencias Forestales. Ref: 5CFE01-060, pag 1-11.

Resumen

La escasez de agua en el suelo es uno de los factores que más influyen en la supervivencia de las plantas en ecosistemas mediterráneos. Cada especie puede mostrar una diferente respuesta a la sequía debido a distintos mecanismos desarrollados para evitar o tolerar el estrés que ésta supone. La estructura física del suelo determina en gran medida el almacenaje potencial de agua y su disponibilidad para las plantas. Así, la compactación moderada del suelo puede tener un efecto relevante en esta respuesta a la sequía. En este trabajo se estudia el comportamiento frente a la sequía de plántulas de 12 especies leñosas presentes en ecosistemas mediterráneos bajo condiciones controladas y en un rango de compactación moderada de suelo. El objetivo principal es conocer cuáles son las características a nivel intra e interespecífico que puedan explicar la diferente respuesta a la sequía. Como objetivo secundario se pretende valorar el papel de los episodios de lluvia estival en el proceso de reclutamiento. La respuesta a la sequía a nivel de transpiración y supervivencia varió enormemente entre las 12 especies estudiadas. En general, considerando todas las especies y dentro de cada especie se observó como una mayor proporción de raíz y menor inversión en hojas aumentaron el tiempo de supervivencia. La compactación del suelo tuvo en general (considerando todas las especies) un efecto positivo sobre el tiempo de supervivencia. Esto puede ser explicado de dos razones: por un lado, un incremento de compactación aumentó la cantidad de agua disponible por unidad de volumen y por otro lado, la compactación afecta a la distribución de biomasa, aumentando la proporción de la raíz y disminuyendo el área foliar. La lluvia estival tuvo un papel amortiguador del estrés por sequía, provocando un retraso de la mortalidad de 80 días, revertiendo sus síntomas en algunas especies, permitiendo el crecimiento y/o el rebrote de plantas muertas.

Palabras clave: densidad aparente, estrés hídrico, distribución de biomasa, sequía, supervivencia, transpiración.

Abstract

Soil water shortage during summer season is one of the main factors that affect plant survival in Mediterranean ecosystems. Each species may show a different response to the summer drought due to specific mechanisms or strategies developed in order to avoid or tolerate it. Soil physical structure determines water storage and availability for plants growth. Thus, moderate soil compaction may play an important role in the drought response. In this work, we study the drought response of seedlings of 12 woody plant species from Mediterranean ecosystem under greenhouse conditions and what influence has a moderate soil compaction on it. Our main objective is to know which plant traits are conditioning the drought response of individuals and among species. A secondary objective is to describe what effect has a summer rainfall on the recruitment process. The drought response in terms of survivorship and transpiration rate were vey different among the 12 species studied. In general, considering all the species together, a higher root proportion and a lower leaf proportion was related to a higher survivorship rate. Moderate soil compaction had a positive effect on survivorship time. It can be explained by two reasons: first, it is related with the compaction effects on water storage, due that a moderate bulk density increment produces a higher water availability per soil volume unit; the other one is related with the soil compaction effect on biomass allocation, due to seedlings had higher root mass ratio and less leaf area in response to moderate soil compaction. The summer rainfall had a buffer effect on drought stress: producing a mortality delay of 80 days, recovering the optimal stage in some species or reactivating the growth of new shoots.

Keywords: biomass allocation, bulk density, drought, survival, transpiration, water stress.

Introducción

La sequía estival es el factor más determinante en el funcionamiento y supervivencia de las plantas en ambientes mediterráneos con el consiguiente efecto sobre la composición de la vegetación (Joffree et al., 1999). Las estrategias particulares de cada especie en respuesta a la sequía han permitido una categorización (Levitt, 1980) más o menos estandarizada, denominando a las especies tolerantes o evitadoras en función al mecanismo o mecanismos fisiológicos empleados para la supervivencia a la sequía. Los mecanismos de tolerancia son aquellos capaces de minimizar las deformaciones y limitaciones que impone el estrés hídrico, llegando a tolerar los daños causados por el mismo para ser reparados a posteriori. Algunos mecanismos de tolerancia serían: aumento de la tasa de transpiración, pérdida de turgor, epinastia, síntesis de sustancias antioxidantes como antocianos, rebrote tras estrés, etc. Sin embargo, las especies evitadoras ajustan su fisiología a la situación de déficit hídrico, intentando controlar las deformaciones que éste provoca. Algunos mecanismos evitadores típicos serían la disminución del área foliar, control estomático, aumento de la densidad radicular para maximizar la toma de agua, etc. Para cubrir todo el espectro de respuestas dado por cada especie y cada individuo concreto, se ha normalizado el uso de diversas medidas fisiológicas que demuestran como ambos mecanismos, evitadotes y tolerantes, no son excluyentes, sino que pueden aparecer en momentos concretos del proceso de resistencia a la sequía. Esto permite completar la definición de cada estrategia específica (Valladares et al., 2008), teniendo en cuenta que la respuesta a la sequía estival supone simultáneamente un complejo de diferentes tipos de estrés: hídrico, térmico y luminoso.

La estructura de una planta puede influir en la resistencia a la sequía. Lloret et al. (1994) encuentran como las especies con mayor proporción de raíz presentaban una mayor supervivencia. Por otro lado, el crecimiento de las distintas especies puede estar asociado a determinadas características funcionales. Así, por ejemplo, se ha descrito como la tasa de crecimiento relativo (RGR) está asociada positivamente a características foliares como el área específica foliar (SLA) y la proporción de hojas (Poorter & Remkes, 1990; Reich & Walters, 1992; Cornelissen et al., 1996; Antúnez et al. 2001; Ruiz-Robleto et al. 2005). Hojas con alto SLA poseen una elevada transpiración que les permiten tener los estomas abiertos y captar CO_2. Sin embargo, esto supone el inconveniente de una mayor pérdida de agua. Por ello, especies con alto SLA y por tanto con alta tasa de transpiración tendrían una mayor susceptibilidad a la sequía. Es por tanto de esperar que a nivel interespecífico existiera un compromiso entre crecimiento (RGR) y supervivencia.

Uno de los factores que indirectamente pueden estar condicionando la respuesta a la sequía es la estructura física del suelo y su consiguiente almacenaje y retención de agua. Una mayor compactación del suelo puede favorecer el contacto raíz-suelo (Arvidsson, 1990), o bien puede impedir la cesión del agua retenida por el suelo por el aumento del potencial matricial (Taylor & Ratliff, 1969; Whalley et al., 2006). Además, las limitaciones al crecimiento de la raíz, y su consiguiente reducción del volumen de suelo explorado, podrían dificultar el acceso al recurso hídrico, lo que convierte a la compactación en un factor indirecto que puede influir en la respuesta a la sequía. Es importante resaltar que los efectos de la compactación sobre la proporción de raíz son muy dependientes de la especie y el rango de compactación estudiado (Alameda & Villar 2009), lo que podría dar lugar a respuestas diferentes ante la sequía.

La precipitación de verano de la Península Ibérica ha mostrado una gran variabilidad interanual en los últimos 50 años, llegando en el sureste a valores de un 75 % de coeficiente de variación (De Luis et al, 2009). Así para el mismo periodo histórico, se ha observado una tendencia en la precipitación de los meses de julio y agosto a aumentar hasta un 20 % en el sur peninsular acompañado de una reducción del 10 % en los meses de junio y septiembre (Mosmann et al., 2004). El panorama dibujado parece seccionar la sequía estival en 2 subperiodos separados por precipitaciones que en algunos casos son muy superiores a la capacidad de absorción del suelo, lo que conlleva erosión y lavado de suelos. Durante estos episodios de lluvias estivales la vegetación puede responder retrasando o evitando la muerte por sequía. Por tanto, la lluvia estival, como factor de retardo de la mortalidad o rescate de supervivientes, podría cobrar relevancia al aumentar la probabilidad de alcanzar la estación húmeda nuevamente.

Los trabajos en condiciones controladas, a pesar de la falta de realismo, pueden permitir una comparación de la respuesta a la sequía sin otros artefactos. En el presente trabajo presentamos la respuesta a la sequía de 12 especies leñosas presentes en ambientes mediterráneos. Se han utilizado distintos tratamientos de compactación moderada del suelo para relacionarlos con cambios en la estructura de las plantas y en el uso del agua del suelo. Se ha estudiado el tiempo de supervivencia, la transpiración (método gravimétrico), área foliar, crecimiento, distribución de biomasa y el empleo de una serie de índices visuales que nos describan el proceso de marchitado y muerte por sequía. También se simuló un episodio de lluvia estival para conocer el efecto sobre la supervivencia.

El presente trabajo tiene como objetivos principales: (i) conocer cuáles son las variables más determinantes en el tiempo de supervivencia a la sequía a nivel inter e intraespecífico; (ii) analizar el papel de la compactación del suelo en dicha respuesta a la sequía; (iii) describir cuáles son las estrategias seguidas por cada especie en respuesta a la aparición del recurso hídrico en el periodo de sequía.

Material y métodos

Este experimento se desarrolló en los invernaderos del Campus Rabanales de la Universidad de Córdoba entre enero de 2006 y junio de 2007. El diseño consta de dos etapas: fase de crecimiento y periodo de sequía. La primera fase duró 205 días aproximadamente, desde enero a agosto de 2006, en la cual las plantas crecieron en condiciones óptimas de agua, luz y temperatura hasta alcanzar un porte similar al del estadío juvenil. El periodo de sequía duró 304 días aproximadamente, desde agosto de 2006 hasta junio de 2007, finalizado cuando todas las plantas habían muerto. En este periodo, el agua se suprimió totalmente, salvo un día de riego (5 de octubre de 2006), con el objetivo de simular un episodio de lluvia estival.

Fase de crecimiento

La media de luz fotosintéticamente activa dentro del invernadero medida en un día despejado (8 de mayo de 2006) fue de 446 ± 89 µmol fotón m^{-2} s^{-1} (media y desviación estándar), con una radiación media diaria a lo largo de todo el periodo de crecimiento de 34.5 mol m^{-2} $día^{-1}$. El agua fue suministrada diariamente mediante sistema de riego por goteo. Para evitar déficits nutricionales se le añadió 1 g de abono de liberación lenta a cada maceta a los 3 meses del inicio (Plantacote Pluss, NPK 14:9:15, Aglukon, Valencia). Las macetas de PVC empleadas tenían una longitud de 50 cm y un diámetro de 10.5 cm (4.33 L). El sustrato se obtuvo mediante una mezcla de arena de río, turba y limo en una proporción 9:6:5, resultando en un suelo de textura arenosa (88% arena, 8% limo y 4% arcilla). Este tipo de suelo es de similares características al encontrado en algunas dehesas de la provincia de Córdoba (Fernández-Rebollo et al., 2004). Los tratamientos de compactación moderada se realizaron manualmente con un émbolo de aluminio consiguiendo un continuo de 8 niveles de compactación creciente con un rango de resistencia a la penetración de 0.1 a 1.0 MPa, o en términos de densidad aparente de 1.12 a 1.28 g cm^{-3}. El número total de muestras fue de 864 distribuidas en: 8 niveles de compactación con 6 réplicas por cada nivel para las 17 especies más un control, donde no se plantó nada.

Las 17 especies leñosas empleadas se escogieron de familias representativas de ecosistemas mediterráneos (Fagaceae: *Quercus ilex*, *Q. coccifera*, *Q. suber*, *Q. canariensis*, *Q. pyrenaica* y *Q. faginea*; Oleaceae: *Olea europaea* y *Fraxinus angustifolia*; Anacardiaceae: *Pistacia lentiscus* y *P. terebinthus;* Caesalpinaceae: *Ceratonia siliqua* y *Cercis siliquastrum:* Rhamnaceae: *Rhamnus alaternus* y *Frangula alnus*) y en otros casos por ser interesantes para el estudio (Rosaceae: *Prunus lusitanica*; Pinaceae: *Pinus sylvestris*; Simaroubaceae: *Ailanthus altissima*).

El análisis de crecimiento se realizó tras 205 días sobre la mitad de las réplicas de cada especie (un total de 432 muestras). Los resultados de esta etapa sobre los efectos de la compactación, parámetros de crecimiento y distribución de biomasa pueden verse en Alameda & Villar (2009) (Capítulo 3).

El área específica foliar (SLA, Specific Leaf Area) de este periodo se usó para el cálculo de área foliar de la etapa de supervivencia.

Fase de supervivencia

Dado que de las 17 especies, 5 de ellas mostraron un alta mortalidad en el primer periodo de la fase de supervivencia (inducido por altas temperaturas), tan sólo se analizarán 12 especies de las citadas anteriormente: *Quercus ilex, Q. coccifera, Q. suber, Q. canariensis, Q. pyrenaica, Q. faginea, Olea europaea, Pistacia lentiscus, Ceratonia siliqua, Prunus lusitanica, Pinus sylvestris y Ailanthus altissima.*

Los valores de temperatura de los 309 días de esta etapa fueron: media 21 ± 5 °C; mínima 14 ± 5 °C y máxima 35 ± 9 °C (valores medios y desviación estándar). La humedad relativa fue de 67 ± 12 %. A los 53 días del comienzo de la sequía se simuló un episodio de lluvia estival mediante un riego a saturación, esto requiere un análisis separado de las 2 subetapas para poder valorar el efecto específico de la lluvia estival.

El seguimiento consistió en: pesar periódicamente las macetas para calcular las pérdidas de agua y calcular la tasa de transpiración; una evaluación visual de los síntomas de estrés hídrico con la consiguiente estima del tiempo de supervivencia; y la retirada de la biomasa seca caída (principalmente hojas) para estimar posteriormente el área foliar. Estos parámetros serían empleados como variables para conocer la estrategia desarrollada por cada especie para la evitación o tolerancia a la sequía. Periódicamente se recogieron las hojas secas de cada individuo para al final tener una estima de la biomasa foliar.

Al final del periodo de sequía se cosecharon todas las plantas muertas para conocer los valores de biomasa (hojas, tallo y raíz). Justo antes de la cosecha se midió la resistencia a la penetración con un penetrómetro (desarrollado por el Departamento de Ingeniería Agroforestal, Universidad de Córdoba), con cono de 12.6 mm de diámetro y 30° de ángulo. También se midió la altura de la maceta para el cálculo de la densidad aparente. Tras la recolección, las distintas muestras (hojas, tallo y raíz) se introdujeron en estufa a

70 °C para obtener la biomasa seca. Se calcularon distintas variables de crecimiento y de distribución de biomasa como: biomasa seca total (BS), fracción de biomasa dedicada a hojas (LMR), fracción dedicada a tallo (SMR), fracción dedicada a raíces (RMR), razón fracción raíz-parte aérea (R/S), tasa de crecimiento relativo (RGR), área foliar total (como Biomasa foliar × SLA) y razón de área foliar (LAR) (Fig. 1) (Villar et al., 2004).

$$\mathbf{LMR} = \frac{\text{BS hojas (kg)}}{\text{BS total (kg)}} \qquad \mathbf{RGR} = \frac{\text{Ln BS total final (mg) - Ln BS total inicial (mg)}}{\text{tiempo transcurrido (días)}}$$

$$\mathbf{SMR} = \frac{\text{BS tallo (kg)}}{\text{BS total (kg)}} \qquad \mathbf{LAR} = \frac{\text{Área total (m}^2\text{)}}{\text{BS total (kg)}}$$

$$\mathbf{RMR} = \frac{\text{BS raíces (kg)}}{\text{BS total (kg)}} \qquad \mathbf{SLA} = \frac{\text{Área total (m}^2\text{)}}{\text{BS hojas (kg)}}$$

Figura 1. Fórmulas de las variables de crecimiento y distribución de biomasa. BS: biomasa seca: BF: biomasa fresca

A efectos comparativos se muestran en el Apéndice suplementario (Apéndice S1) los valores medios por especie de algunas variables estudiadas en las dos etapas del experimento: crecimiento (Cap. 3) y supervivencia (Cap. 4). En general, se observa un incremento de la proporción de raíz (RMR), una disminución de la proporción de hojas (LMR) y un ligero incremento de la biomasa total en el experimento de sequía comparado con el experimento de crecimiento. La densidad aparente del suelo es similar en los dos experimentos, salvo en *Ailanthus altisima* que puede ser debido a un efecto de-compactador de esta especie. En cambio, la resistencia a la penetración es mucho mayor en el experimento de sequía, debido a la disminución del porcentaje de agua, que aumenta la resistencia a la penetración.

Tasa de supervivencia e índices visuales de estrés hídrico

A partir del tiempo de tiempo de supervivencia obtenido mediante el seguimiento semanal se calculó la tasa de supervivencia de cada especie. Para describir qué parámetros son los más determinantes sobre el tiempo de supervivencia se realizaron correlaciones a nivel intraespecífico con todas las variables estudiadas, tanto de crecimiento como edáficas. Se calculó también el t_{50} definido como el tiempo en el que la supervivencia de cada especie era un 50% del total de muestras.

Para estimar el grado de estrés a lo largo de la fase de sequía se definieron 7 índices visuales distintos, categorizando el estado de cada planta en el tiempo (Tabla 1). Estos índices permitirían describir la dinámica de respuesta para cada especie en función a su capacidad para prolongar el estado óptimo (índice 1), o por el contrario por la rapidez en alcanzar la muerte de la totalidad de la fracción aérea (índice 7). Por otro lado, la dinámica de paso de unos índices a otros nos agruparía las especies en función a su estrategia de respuesta a la sequía, según el número de estados por los que pasasen y el tiempo empleado en cada uno.

Tabla 1. Descripción de los índices visuales empleados para los síntomas de estrés hídrico.

Índice visual	Descripción	% hojas muertas
1	Viva: toda la parte aérea en estado óptimo	0
2	Viva pero con leves síntomas: pérdida de turgencia, cambios de color, aparición de manchas.	casi 0
3	Estresada: marchitado leve	<<50
4	Estresada: marchitado medio	50
5	Estresada: marchitado generalizado	>>50
6	Casi muerta: conserva algunas hojas elásticas, parcial o totalmente	casi 100
7	Muerta: toda la parte aérea seca	100

Cálculo de la tasa de transpiración

Para el cálculo de la tasa de transpiración se llevó a cabo un seguimiento de las pérdidas de peso de todas las macetas mediante una balanza con precisión de 1 g. La frecuencia de pesada fue disminuyendo a medida que las pérdidas se hacían más pequeñas. Durante el periodo de sequía el sistema planta-maceta perdió peso debido a: evaporación de suelo, evapotranspiración de planta y pérdidas gravitacionales. La cantidad de agua perdida por gravedad y evaporación se consideró constante para todas las macetas y de valor despreciable. Así que no se tuvo en cuenta para el cálculo de la transpiración, esto puede suponer una sobreestimación de la transpiración, pero con un error similar para todas las macetas. Para conocer la cantidad de agua presente en cada pesada, se empleó un predictor que define la capacidad del suelo, construido entre una submuestra de macetas saturadas hasta su capacidad de campo y posteriormente llevadas a desecación total. Así, obtuvimos una ecuación de regresión (Suelo seco = -494.23 + 0.8951× Suelo a capacidad de campo; $r^2 = 0.99$; $P = 0.0000$) que representa la relación entre el peso húmedo de suelo y su correspondiente cantidad de suelo seco. De esta forma se calculó el peso seco de suelo de cada maceta y por diferencia con el peso en un determinado tiempo se calculó la cantidad de agua. Posteriormente estudiamos la dinámica de pérdida absoluta de agua con respecto al tiempo, obteniendo una ecuación de regresión cuya pendiente nos determinaría la tasa de transpiración expresada en gramos de agua perdidos por día.

Resultados

Factores determinantes de la supervivencia

De forma general, considerando todas las especies de forma conjunta, las variables que influyen negativamente en el tiempo de supervivencia son: biomasa, tasa de crecimiento relativo (RGR, *Relative Growth Rate*), proporción de hojas (LMR, *Leaf Mass Ratio*), proporción de tallo (SMR, *Stem Mass Ratio*), área foliar, y razón de área foliar (LAR, *Leaf Area Ratio*) (Tabla 2). Sin embargo, la proporción de raíz (RMR, *Root Mass Ratio*) y la densidad aparente del suelo parecen prolongar el tiempo de supervivencia a la sequía (Tabla 2).

A nivel intraespecífico, la respuesta resulta más heterogénea, aunque en general se siguen viendo las mismas tendencias. En cuanto a la biomasa total, mientras que para 4 de las 12 especies (33 %) la biomasa produce un efecto negativo sobre la supervivencia, para 2 de ellas, *Ceratonia siliqua* y *Pistacia lentiscus*, el incremento de biomasa parece tener un efecto positivo de aumento de la supervivencia. En las otras variables estudiadas si se encuentran un patrón parecido al patrón general. Así, la proporción de hojas y tallo (LMR y SMR, respectivamente) producen de forma consistente un efecto negativo sobre la tasa de supervivencia. Para un total de 7 de las 12 especies (58 %) el poseer una mayor proporción de hojas está relacionado negativamente con la supervivencia, acompañado en 3 de ellas de un efecto negativo de la proporción de tallo (Tabla 2). Para 6 especies (un 50%) (todas del género *Quercus*) el área foliar tiene un efecto negativo sobre la supervivencia. De igual forma, un aumento de la razón de área foliar (LAR, variable que integra el área foliar de la planta con respecto a la biomasa total) estuvo relacionado con una disminución de la supervivencia en un total de 7 especies (58 %).

Tabla 2. Correlaciones entre el tiempo de supervivencia y las variables de estudio para cada especie y en conjunto. N: tamaño muestral; RGR: tasa de crecimiento relativo; LMR: proporción de hojas; SMR: proporción de tallo; RMR: proporción de raíz; LAR: razón de área foliar. La significación se indica mediante: a: 0.05 < P < 0.10; *: P < 0.05; **: P < 0.01; ***: P < 0.001.

sp	N	Biomasa	RGR	LMR	SMR	RMR	Área foliar	LAR	Tasa de transpiración	Densidad aparente
Quercus ilex	22	--	--	-0.66 **	-0.38 a	0.67 ***	-0.42 *	-0.66 **	--	--
Q. coccifera	22	--	--	-0.53 *	--	0.49 *	-0.51 *	-0.53 *	--	--
Q. faginea	22	-0.50 *	--	-0.67 **	-0.80 ***	0.78 ***	-0.75 ***	-0.67 **	--	--
Q. canariensis	22	-0.57 **	-0.48 *	-0.51 *	-0.58 **	0.60 **	-0.70 ***	-0.51 *	-0.69 ***	--
Q. pyrenaica	23	--	--	--	--	--	-0.43 *	--	-0.45 *	--
Q. suber	24	-0.53 **	--	-0.75 ***	-0.64 **	0.80 ***	-0.74 ***	-0.75 ***	0.53 **	--
Ailanthus altissima	21	-0.53 *	--	--	--	--	-0.42 a	--	0.60 *	--
Pinus sylvestris	22	--	--	--	--	--	--	--	--	--
Prunus lusitanica	23	--	--	-0.68 ***	--	0.65 **	--	-0.68 ***	--	0.436 *
Olea europaea	23	--	--	--	--	0.44 *	--	--	--	--
Ceratonia siliqua	23	0.45 *	--	-0.71 ***	-0.38 a	0.70 ***	--	-0.71 ***	--	--
Pistacia lentiscus	15	0.56 *	0.45 a	-0.51 a	--	--	0.46 a	-0.51 a	--	--
Todas	262	-0.46 ***	-0.22 ***	-0.33 ***	-0.29 ***	0.40 ***	-0.47 ***	-0.24 ***	--	0.33 ***

Sin embargo, una mayor proporción de raíz (RMR) supuso para el 66 % de las especies estudiadas un aumento de la supervivencia.

El efecto de la tasa de transpiración sobre la supervivencia parece ser muy dependiente de la especie que consideremos. Así, para *Quercus canariensis* y *Q. pyrenaica* el aumento de la transpiración parece disminuir el tiempo de supervivencia, siendo contraria la respuesta en *Q. suber* y *Ailanthus altissima*, donde la transpiración está asociada positivamente al tiempo de supervivencia.

Por último, la compactación del suelo medida en términos de densidad aparente tan sólo muestra un efecto significativo y positivo para la especie *Prunus lusitanica*. Este efecto aislado contrasta con la respuesta general de todas las especies, en la que se observa cómo el aumento de la densidad aparente parece aumentar el tiempo de supervivencia.

Una explicación para el efecto positivo que la compactación moderada puede tener de forma general sobre la supervivencia a la sequía podría estar relacionada con la disponibilidad de agua en el suelo. Un incremento leve de la compactación supone una disminución del volumen de aire con un aumento de la masa de suelo. Si relacionamos la densidad aparente con la cantidad de agua almacenada (que denominamos disponibilidad de agua), observamos que hay un incremento de ésta con la densidad aparente (Fig. 2 a). Esto puede ser debido a que la compactación moderada produce una disminución de la macroporosidad aumentando el porcentaje de microporos, incrementando la capacidad de retención de agua con la compactación moderada (Fig. 2b).

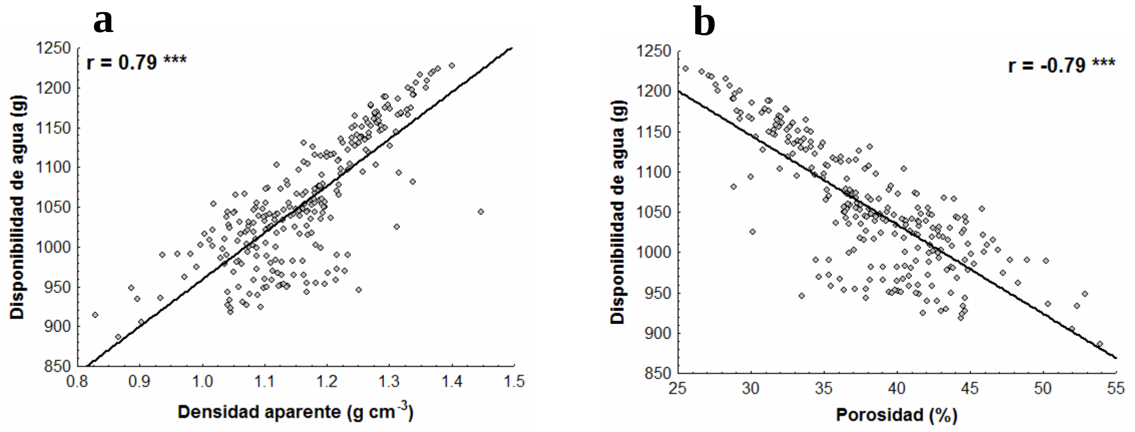


Figura 2. Relación entre disponibilidad potencial de agua y la densidad aparente (a) y porosidad (b). Se muestra el valor del coeficiente de regresión (r) y su significación con asteriscos (ver tabla 2).

El papel de la compactación moderada del suelo

De forma general, considerando todas las especies a la vez, la compactación del suelo tiene un efecto negativo sobre biomasa, RGR, SMR, área foliar y LAR. Mientras que ejerce un efecto positivo sobre RMR y la tasa de transpiración (Tabla 3). Se observa por tanto como el efecto general de la compactación es a aumentar la proporción de raíz (RMR, Fig. 3a), junto con un descenso en el área foliar (Fig. 3b). Estos dos cambios podrían estar relacionados con el incremento en el tiempo de supervivencia que se observa con la compactación moderada.

Tabla 3. Correlaciones entre la densidad aparente del suelo y las variables de estudio para cada especie y en conjunto. N: tamaño muestral; RGR: tasa de crecimiento relativo; LMR: proporción de hojas; SMR: proporción de tallo; RMR: proporción de raíz; LAR: razón de área foliar. La significación se indica mediante: a: 0.05 < P < 0.10; *: P < 0.05; **: P < 0.01; ***: P < 0.001.

sp	N	Biomasa	RGR	LMR	SMR	RMR	Área foliar	LAR	Tasa de transpiración
Quercus ilex	22	--	--	--	-0.41 a	0.36 a	--	--	--
Q. coccifera	22	0.49 *	0.59 **	--	--	--	0.53 *	--	0.60 **
Q. faginea	22	--	--	--	--	--	--	--	0.62 **
Q. canariensis	22	--	--	-0.38 a	--	--	--	-0.38 a	0.48 *
Q. pyrenaica	23	--	--	0.43 *	--	-0.40 a	0.41 a	0.43 *	0.39 a
Q. suber	24	--	--	--	--	--	--	--	0.67 ***
Ailanthus altissima	21	--	--	--	--	--	--	--	0.60 **
Pinus sylvestris	22	--	--	--	--	--	--	--	0.60 **
Prunus lusitanica	23	--	--	--	--	--	--	--	--
Olea europaea	23	0.42 *	--	--	--	--	--	--	0.77 ***
Ceratonia siliqua	23	0.43 *	--	--	--	--	--	--	0.76 ***
Pistacia lentiscus	15	--	--	--	--	--	--	--	0.73 **
Todas	262	-0.38 ***	-0.19 **	-0.11 a	-0.13 *	0.15 *	-0.42 ***	-0.23 ***	0.54 ***

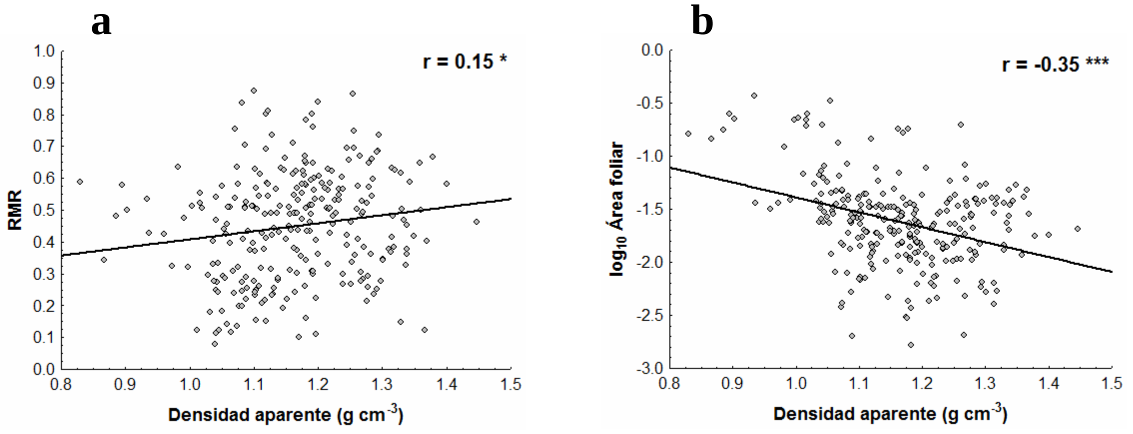


Figura 3. Relación entre densidad aparente y (a) la proporción de raíz (RMR) y (b) área foliar (log transformada). Se muestra el valor del coeficiente de regresión (r) y la significación en asteriscos (ver tabla 3).

Mecanismos de respuesta a la sequía: la tasa de transpiración

Se observó como en general las pérdidas de agua fueron fuertes al principio de someter las plantas a la sequía (días 1 a 10; Fig. 4) y fueron disminuyendo progresivamente (días 10 a 20 y días 20 a 30). Es de destacar las bajas tasas de transpiración de *Pinus sylvestris* y el fuerte control de la transpiración de

Olea europaea, que son dos de las especies con mayor tiempo de supervivencia.

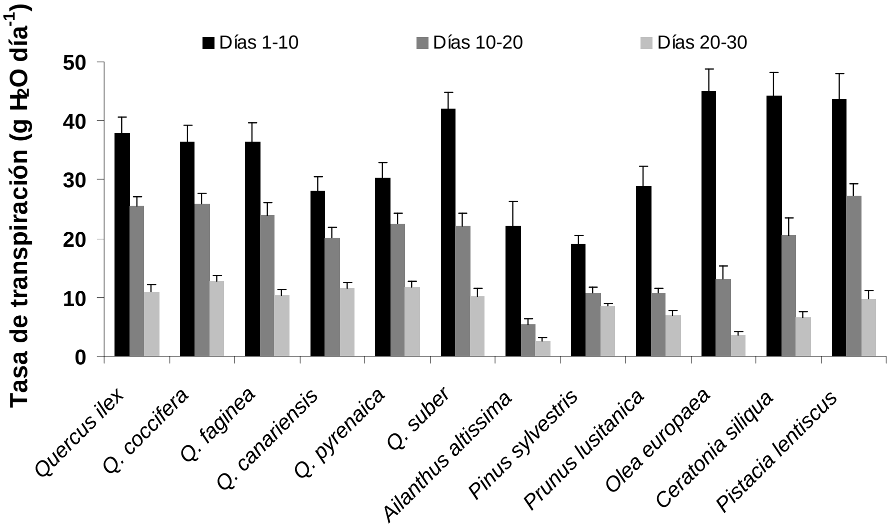


Figura 4. Tasa de transpiración media por especie en los 30 primeros días desde el inicio de la sequía. Se muestra el valor medio más el error estándar en intervalos de 10 días.

Por otro lado, como se observó en la tabla 3, la compactación estuvo relacionada positivamente con la densidad aparente, tendencia que se encuentra para los distintos periodos de transpiración medidos (Fig. 5). Esto puede ser debido a lo que se comentó anteriormente, ya que un incremento de la densidad aparente estaba asociado a una mayor cantidad de agua en la maceta y por tanto una mayor reserva de agua, que podría usarse en la transpiración.

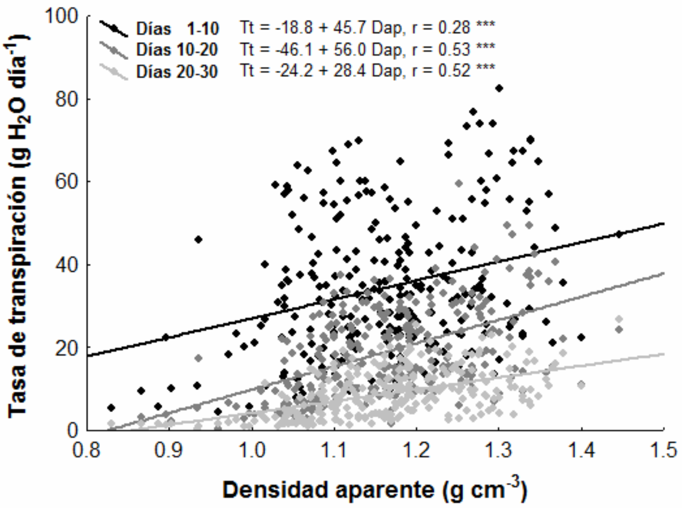


Figura 5. Relación entre la densidad aparente y la tasa de transpiración en tres periodos sucesivos. Se muestra la ecuación de regresión para cada periodo y el valor del coeficiente asociado (r).

Dinámica de mortalidad y el efecto de la lluvia estival

Podemos analizar también la respuesta de la población total ordenando las especies por su t_{50} (tiempo en el que la mitad de la población muere) (Fig. 6), lo que nos da una idea de la capacidad de resistencia entre las diferentes especies, aunque también puede estar debido al tamaño de la plántula.

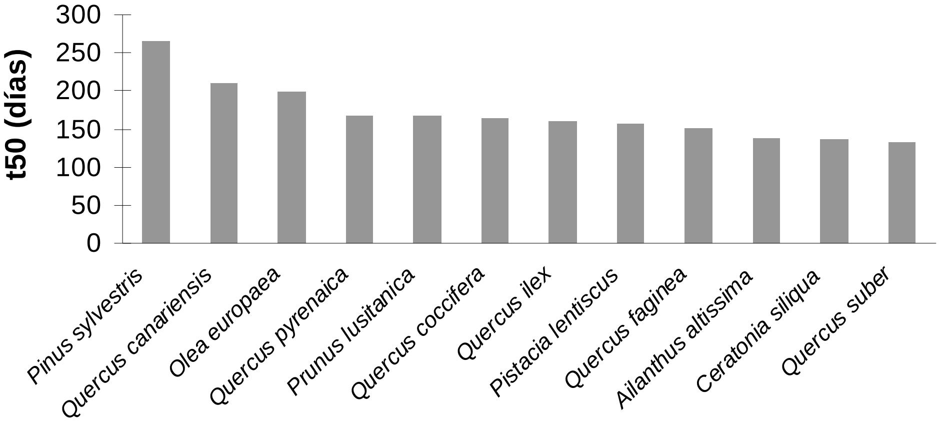


Figura 6. Tiempo medio por especie para el que el 50 % de la población estaba muerta.

En general, las especies con una menor supervivencia son *Q. suber*, *Ceratonia siliqua* y *Ailanthus* (con alrededor de 150 días), mientras que la especie con un mayor tiempo de supervivencia fue *Pinus sylvestris* (con un tiempo de 250 días). Contrasta el comportamiento de *Q. canariensis*, que es una especie localizada en sitios húmedos, y por tanto debería tener un tiempo de supervivencia bajo. Esta incongruencia podría ser debida al pequeño tamaño de las plátulas de esa especie.

Es interesante observar además la dinámica de supervivencia con el tiempo para cada especie, así como el efecto del riego, simulando una lluvia estival (Fig. 7). En general, para todas las especies, la lluvia estival determinó una estabilización de los porcentajes de supervivencia (Fig. 7), y constituyó un tiempo cercano a los 80 días en que la mortalidad cesó. También para algunas especies, la lluvia estival tuvo un efecto leve sobre el aumento del número de supervivientes de la población total, es decir por rebrote de la parte aérea. Sin embargo, la capacidad de rebrote fue heterogénea para las distintas especies. Por ejemplo, *Quercus suber* pasó de un 60 % de supervivencia a un 75%, y para *Ailanthus* el incremento fue mucho mayor de un 20 % a un 80 %.

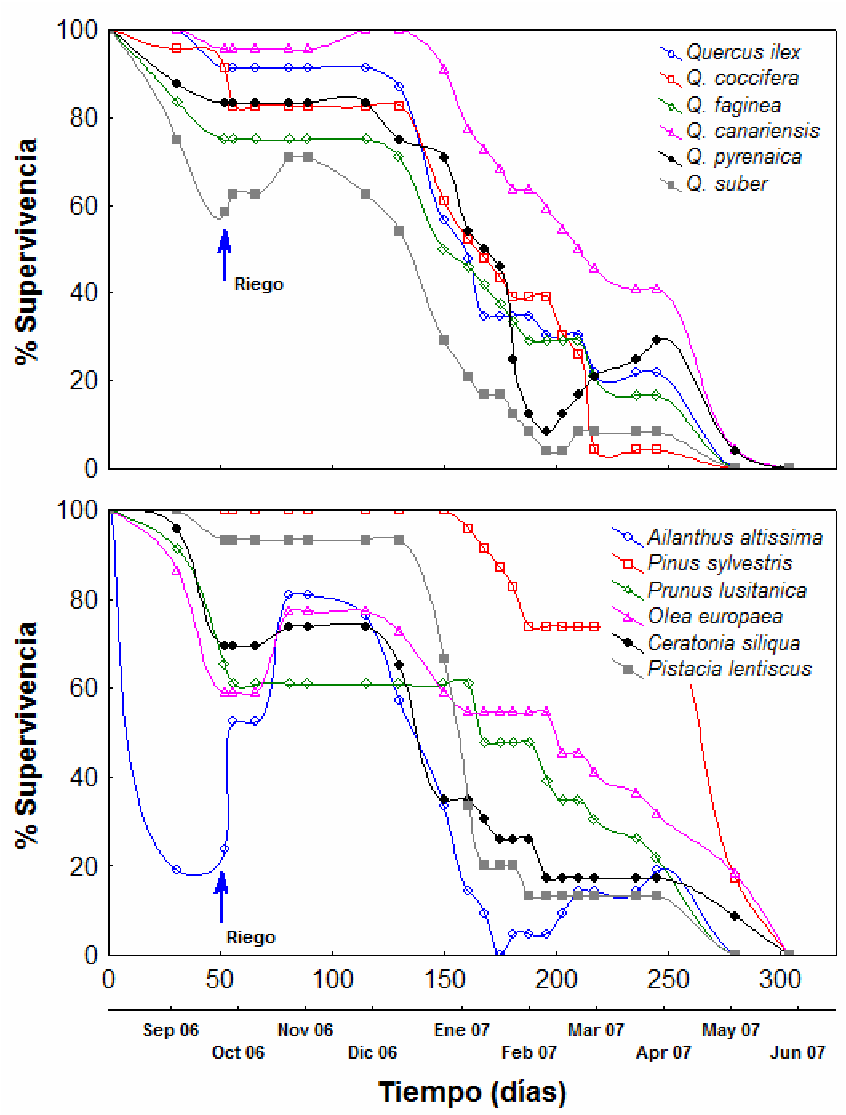


Figura 7. Porcentaje de supervivencia a lo largo del tiempo para las 12 especies estudiadas. La flecha indica el momento del riego simulando una lluvia estival con el objetivo de evaluar el efecto sobre el tiempo de supervivencia.

El uso de de los índices visuales es muy interesante ya que nos describe diversas estrategias seguidas ante la sequía y ante la reaparición del recurso hídrico (Fig. 8). Puede observarse como las distintas especies presentan una dinámica distinta. Por ejemplo si comparamos *Q. suber* con *Q. ilex*, podremos observar como en *Q. ilex* la mayoría de las plantas (60 %) están sin síntomas aparentes hasta el día 120, mientras que para *Q. suber* al cabo de 30 días, la mayoría (60 %) muestran cierto grado de marchitez.

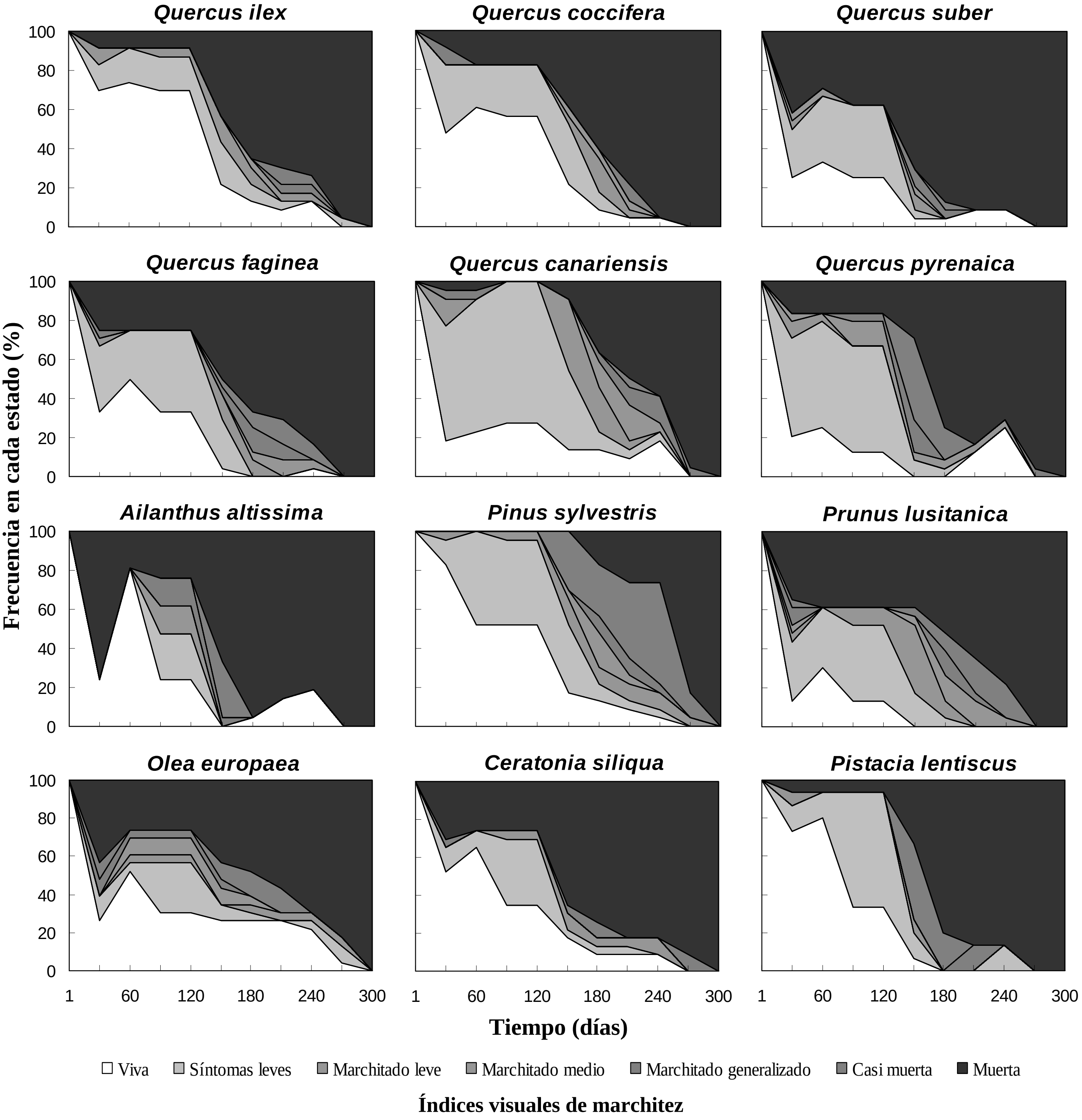


Figura 8. Evolución del estado de marchitez a lo largo del tiempo para las 12 especies estudiadas. En ordenadas se muestra el porcentaje de individuos para cada estado de marchitez, estimado mediante índices visuales (ver tabla 1).En el día 53 se sometió a las especies a un riego a saturación.

Podríamos clasificar la respuesta a la lluvia estival en distintos grupos. Así, vemos:

- Reversión de síntomas de estrés: el efecto más evidente de la lluvia fue la recuperación de la turgencia. En el caso de especies como *Pistacea lentiscus* que ante el estrés por sequía muestran un enrojecimiento por antocianos, tras la lluvia estival se observó un reverdecimiento total de las hojas (Fig. 9).
- Reactivación del crecimiento: para especies que no habían acusado en exceso la etapa de sequía se observó la aparición de nuevos brotes.
- Reclutamiento de individuos muertos: al considerar a los individuos muertos sólo por su porción aérea se ignora si la raíz sigue viva o si sería capaz de volver a generar nuevamente una planta completa. En el caso de algunas especies tras el riego comenzaron a rebrotar de nuevo (*Quercus suber*, *Ailanthus*) o emitieron nuevos vástagos desde la raíz (*Ailanthus*).

Figura 9. Muestra del efecto de la lluvia estival: reverdecimiento de Pistacia lentiscus (izda.), nuevo crecimiento en Quercus canariensis (centro) y rebrote en Ailanthus altísima (dcha.).

Discusión

En este trabajo se ha observado como la respuesta a la sequía ha variado enormemente entre las 12 especies estudiadas. Estos resultados son esperables teniendo en cuenta que las especies tienen una distribución muy distinta. Sin embargo, a pesar de esas diferencias, podemos establecer algunas conclusiones generales. Por un lado, tanto considerando todas las especies como a nivel intraespecífico, se observó como una mayor proporción de raíz y menor inversión en hojas aumentaron el tiempo de supervivencia. Resultados similares han sido encontrados por Lloret et al. (1994) de forma que las especies con mayor proporción de raíz presentaban una mayor supervivencia.

Por otro lado, uno de los factores que indirectamente pueden estar condicionando la respuesta a la sequía es la estructura física del suelo. Así, hemos observado como la compactación del suelo tuvo en general (considerando todas las especies) un efecto positivo sobre el tiempo de supervivencia. A priori, se podría pensar que la compactación podría tener un efecto negativo sobre la supervivencia, ya que por ejemplo se produce una disminución en la elongación de la raíz (Taylor & Ratliff, 1969). Las limitaciones al crecimiento de la raíz y su consiguiente reducción del volumen de suelo explorado, dificultarían el acceso al agua, lo que convierte a la compactación en un factor indirecto que puede influir en la respuesta a la sequía. Sin embargo, hay que tener en cuenta que el rango de compactación en este trabajo es moderado. Los efectos de la compactación pueden variar de forma drástica en relación al rango de estudio (Alameda y Villar, 2009). En este trabajo hemos observado cómo un incremento de la compactación aumentó la cantidad de agua en la maceta, presentando por tanto una mayor reserva de agua. Fleming et al. (2006) mostraron cómo la compactación del suelo puede tener un efecto positivo sobre la supervivencia. Esto está principalmente determinado por la eliminación de los efectos negativos de la macroporosidad, relacionado a su vez con la pérdida de retención de agua. Por otro lado, la planta puede ver modificada su arquitectura con la compactación, como por ejemplo un aumento de la proporción de raíz y una disminución del área foliar. Estos cambios pueden favorecer una mayor supervivencia. A esto se añade que una compactación moderada puede favorecer el contacto raíz-suelo (Arvidsson, 1990) y aumentar la absorción de agua.

También sería importante resaltar el compromiso que se establece entre crecimiento y supervivencia. Por un lado, la tasa de crecimiento relativo está influida positivamente por el área foliar y la proporción de hojas y tallo, pero negativamente con la proporción de raíz. En cambio, la relación de estas variables con el tiempo de supervivencia es la contraria. Así, un mayor área foliar, proporción de hojas y tallo está relacionado con una menor tasa de supervivencia. En cambio, una mayor proporción de raíz esta asociado a una mayor tasa de supervivencia.

La precipitación de verano de la Península Ibérica ha mostrado una gran variabilidad interanual en los últimos 50 años. Esto hace que con cierta frecuencia aparezcan veranos lluviosos que podrían tener consecuencias en el establecimiento de la plantas. La lluvia estival experimental ha tenido un papel amortiguador del estrés por sequía, provocando un retraso de la mortalidad de 80 días. Sería esperable que este lapso de tiempo permitiese alcanzar la estación húmeda nuevamente.

Por último, el empleo de los índices visuales nos ha permitido conocer la estrategia seguida por cada especie en respuesta a la sequía y a la lluvia estival. Se comprueba que cada especie muestra un comportamiento diferente ante el mismo evento de lluvia, lo que podría tener consecuencias en la distribución de las especies y la composición de las comunidades.

Conclusiones

La respuesta a la sequía a nivel de transpiración y supervivencia ha diferido enormemente entre las 12 especies estudiadas. De forma general, una mayor proporción de raíz y menor inversión en hojas aumentaron el tiempo de supervivencia. La compactación del suelo considerando todas las especies tuvo un efecto positivo sobre el tiempo de supervivencia. Esto pudo ser debido a que un incremento de la compactación aumentó la cantidad de agua en la maceta al disminuir la macroporosidad; y por otro lado, a que una respuesta de la plantas a la compactación moderada fue aumentar la proporción de raíz y disminuir el área foliar. La lluvia estival tuvo un papel amortiguador del estrés por sequía, revertiendo sus síntomas, permitiendo el crecimiento y/o el rebrote de plantas muertas.

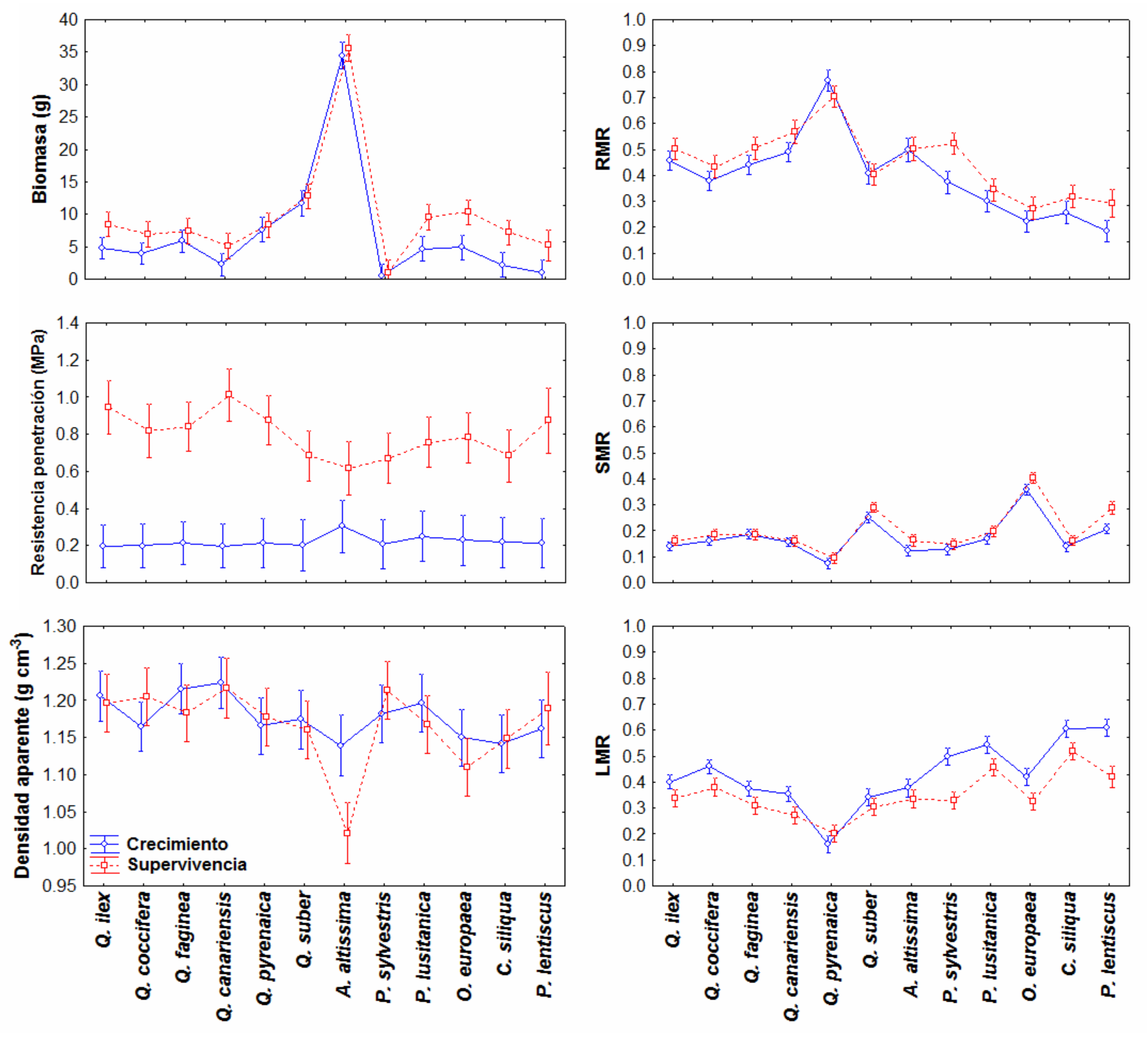
Agradecimientos

Agradecemos a Emilio González su gran trabajo y esfuerzo en el montaje del sistema de riego para la simulación de la sequía. A Álvaro Alameda, Ángel

Ocaña y Kris Calero por su colaboración durante la difícil cosecha de raíces. A Pepón Vacas por las medidas de resistencia a la penetración. Gracias a Paco Conde por su ayuda en distintas fases de la toma de datos. Agradecemos el préstamo del penetrómetro y los consejos de su utilización a Juan Agüera. Este experimento ha sido financiado por el proyecto DINAMED (CGL2005-05830-C03- 02) e INTERBOS (CGL2008-04503-CO3-02) y con fondos FEDER. Este experimento se realizó en los invernaderos de la Universidad de Córdoba (Servicio Centralizado de Apoyo a la Investigación).

Apéndice suplementario

Apéndice S1. Valores medios por especie de algunas variables estudiadas en las dos etapas del experimento: crecimiento (Cap. 3) y supervivencia (Cap. 4). RMR: proporción de raíz. SMR: proporción de tallo. LMR: proporción de hojas.



Chapter 5

Plants responses to soil compaction under shade, low water, and mechanical stress interaction – A *Nicotiana tabacum* essay

Manuscript in preparation

Abstract

It is well established that soil compaction has a negative effect on plant development; by limiting to the root elongation rate, modifying plant-soil relations or in general reducing plant growth. However, are we sure that these effects are constant? Are they independent of other ambient factors?

The disturbances that commonly produce compaction in the soils do not only modify soil structure. It would expect to find out watering heterogeneity moreover soil compaction during agriculture labors, or it would be easy to detect mechanical stress during animal grazing and trampling; or even in the forestry clearcut we could modify light and wind conditions and exposure. Therefore, soil compaction can interact with other ambient factors very relevant for plants. This work investigates soil compaction interactions with 3 important factors for plant development: light, water and mechanical stress. To this end we exposed tobacco plants (*Nicotiana tabacum L.*) under greenhouse conditions to a wide soil compaction range and 4 different treatments: shade, low water, mechanical stress and control conditions. To measure these interactive effects we analyzed plant growth and biomass allocation and root morphology and anatomy. The results showed a strong trade-off between soil compaction and these abiotic factors. In control conditions plants exhibited a binomial response model with positive and negative effects; compaction under shade produced no effects; and under low water and mechanical stress plants only manifested negative effects. In conclusion, this work underling that soil compaction has quantitative effects highly dependent on the ambient conditions which it is produced with.

Keywords: GLM, root anatomy, root morphology, relative growth rate.

Resumen

Es de sobra conocido el efecto negativo que la compactación del suelo tiene sobre el desarrollo de las plantas: limitando la elongación de la raíz, modificando las relaciones planta-suelo y produciendo en términos generales una reducción del crecimiento. Sin embargo, ¿estamos seguros de que dicho efecto sea constante? ¿Es independiente de otros factores ambientales? Las perturbaciones que normalmente producen compactación en el suelo no modifican únicamente la estructura física de éste. Es esperable encontrar que durante el laboreo agrícola que produce compactación se produzcan a la vez riegos heterogéneos, que durante el pisoteo de animales se genere un cierto estrés mecánico, o que durante las labores de aclareo forestal modifiquemos las condiciones de luz y exposición al viento. Es decir, la compactación puede interactuar con otros factores ambientales de gran relevancia para las plantas. Este trabajo estudia las interacciones entre la compactación del suelo y 3 factores importantes para el desarrollo vegetal: luz, agua y estrés mecánico. Para ello se sometieron plántulas de tabaco (Nicotiana tabacum L.) en condiciones de invernadero a un rango amplio de compactación de suelo bajo 4 tratamientos distintos: sombra, bajo régimen hídrico, estrés mecánico y condiciones control. Para estudiar los efectos de estas interacciones se analizó el crecimiento, distribución de biomasa, morfología y anatomía radicular. Los resultados de la compactación mostraron un elevado grado de compromiso con estos factores abióticos. En condiciones control las plantas manifiestan un modelo de respuesta a la compactación binomial, de efectos positivos y negativos; bajo sombra la compactación no produce efecto alguno; mientras que en condiciones de bajo riego y estrés mecánico las plantas sólo responden de forma negativa. En conclusión, este trabajo pone de manifiesto como la compactación del suelo es un factor de efectos cuantitativos y dependientes de las condiciones ambientales en las que se produzca.

Palabras clave: *anatomía radicular, GLM, morfología radicular, tasas de crecimiento relativo.*

Introduction

Soil compaction is commonly considerd to have a negative effect on plant growth and agricultural yields (Wolkowsky, 1990; Hansen, 1996; Kozlowsky, 1999). It is also considered to be an ecological factor that for instance plays a role in hampering forest succession after land clearance for pasture use (Laurence 2003). But soil compaction may also have positive effects on plant growth depending on: soil type (Gómez et al., 2002; Whalley et al., 2008), range studied (Alameda and Villar, 2009) and the species considered (Godefroid and Koedam, 2004). Williamson and Neilsen (2000) showed that the relative influence of compaction could differ considerably depending on the forest site being considered.

The direct impact of soil compaction is an increase of the mechanical resistance to root penetration, thus making more difficult for plants to exploit a large soil volume. But soil compaction may interact with other environmental factors as well. For example, it may increase the soil matrix potential (Whalley et al., 2006), thus reducing water availability to plants (Taylor and Ratlif, 1969). In field conditions, it has also been shown how soil compaction is related to grazing (Drewry et al., 2008), clearcut harvesting in forest management (Small and McCarthy, 2002) or tillage in agricultural system (Diaz-Zorita et al., 2002), all of which tend to increase light availability as well wind exposure. Overall this raises the question as to the extent to which the effects of soil compaction depend on light and water availability as well the magnitude of the external forces (i.e. wind loading) to which plants are exposed. To the best of our knowledge this question has not been addressed.

Light is clearly the most important factor affecting plant growth; it has ecological implications on species evolution, on their competitive ability, adaptability and general development. High light availability tends to increase the demand of plants for soil resources, and plants at high light availability should thus be expected to me susceptible to changes in soil compaction. Water availability could be ranked the second most relevant factor to plant growth. Heterogeneity in water availability can strongly determine plant species distributions. However, the effects of water availability in plants are strongly determined by the physical characteristics of the soil. Cortina et al. (2008) addressed how under natural conditions it is difficult to separate the effects of water limitations from other physical factors affecting root growth. Soil compaction entails changes in various soil properties such as penetration resistance, porosity and bulk density. Since these properties have different and interactive effects in plants, controlled greenhouse and lab experiments are needed in which they can be quantified separately.

Plants typically respond to mechanical stress through reduced stem elongation and increased allocation to root growth, response commonly denoted as thigmomorphogenesis (Jaffe and Forbes, 1993; Anten et al., 2005). It has also been suggested that responses to soil compaction (i.e., mechanical impediment to root growth) and externally applied mechanical force (wind, flexing or rubbing) on the plant shoot involve at least partly the same signal transduction pathway (Anten et al., 2006). It could thus be hypothesized that externally applied mechanical stress may aggravate the effects of soil compaction.

Godefroid and Koedam (2008) suggested that silvicultural treatment can be viewed as a disturbance in the ecological sense, where a pool of factors co-occurred (changes in soil compaction, light and water availability, wind, etc) at the same time, all of which has biological relevancy. Therefore, are we sure when soil compaction is mainly assigned the role of a negative factor? Would it be true in all conditions? Could change the effects of soil compaction depending of other environmental factors?

In general, soil compaction is studied with regards to its effects on overally plant growth. While this provides an indications of the degree to which soil compaction affects plant performance, it does not give insight into the underlying mechanisms. Plant responses to soil compaction are probably in the first place mediated by changes in root characteristics and functioning. It is commonly reported that specific root length (SRL; root length per unit root mass) decreases with soil compaction (Bengough and Mullins, 1990). Atwell (1993) suggested this to be an effect of mechanical impedance, because of longitudinal growth is reduced in favor of radial one. These morphological distortions are resulted of changes on cells division axis and proliferation rate during root elongation (Dexter, 1987). It has been suggested that activity of the phytohormones ABA and ethylene are involved in regulating responses to compaction (Hussain et al., 2000; Roberts et al., 2002; Anten et al., 2006; Whalley et al., 2006), having two main effects: changes in root morphology and shoot growth inhibition. Although plants are able to regulate shoot growth in response to a mechanical impedance signal, it is difficult to avoid side effects on water uptake. Due to compaction, root length and the amount of fine roots are reduced, while soil matrix potential is increased; both of which reduce the ability of plants to take up water. A root diameter decrease has been suggested (Hund et al., 2009) in response to water stress. This is coherent with the theoretical considerations about hydraulic conductivity and resistance (Steudle, 2000), which assumes that roots with a smaller cortex width have lower resistances for water transport from soil to xylem vessels. When roots in secondary growth have been considered, anatomy may change with a xylem vessels diameter decrease in order to maximize hydraulic conductivity under safety margins (Martínez-Vilalta et al., 2002). Therefore, we would expect anatomic changes in response to water stress under compaction conditions in two directions: cortex reduction in fine roots on primary growth and xylem vessels diameter reduction in roots on secondary growth.

In this paper we address the following questions: (i) what are the effects of soil compaction on plant performance, (ii) to what extent do these effect depend on light and water availability and on the degree of mechanical stress to which plants are exposed and (iii) to what extent can the effects on growth be explained by changes in root characteristics? To this end we exposed tobacco plants (*Nicotiana tabacum*) to different levels of compactions, light and water availability and mechanical stress. Tobacco is one of the most extended agriculture species with a high economic value; there is a few reference testing tobacco responses to soil compaction (Tursic et al., 2008); and is used as a model system in many physiological studies on light, water and mechanical responses of *Nicotiana ssp*. Effects of soil compaction in combination with other environmental factors have not been studied.

Materials and methods

Growth facility and plant material

This experiment was carried out in a greenhouse at Utrecht University (The Netherlands) between June and August of 2009. We used tobacco (***Nicotiana tabacum*** L., cv. Samson NxN), which is an annual plant, with this particular cultivar reaching a height of about 2 m. On May 18th, seeds were sown in trays in a mixture of sand and potting soil. Seedlings were grown at 30% of natural daylight, a level achieved with neutral-density shade cloth and shading by the greenhouse roof. When plants reached a fresh mass of 1.4 g ± 0.6 g, they were transplanted into PVC tubes of 30 cm in height and 10.5 cm in diameter. Pots base were perforated with 5 orifices of 5 mm diameter to allow a good drainage. For the description of soil type and treatment see below.

The light level during the experiment was 50% of natural day light (300 µmol photon m^{-2} s^{-1}) and was created by the shading of the greenhouse roof. The day/night temperatures in the greenhouse were set to 22 and 18 °C, respectively. Watering was done by hand using 100 ml each time and 3 times a week until a total amount of 2900 ml during 70 days of growing stage. This system forces plants to use soil water reserve as a function of soil compaction treatment.

Experimental design: water, light and mechanical treatments

The experiment was set up as an incomplete factorial design. There were three soil compactions treatments (NC, no compaction; MC, medium compaction; HC, high compaction). Within each compaction level plants were assigned to one of four additional treatments: CO, used as control, plants were grown in optimal conditions of light, water and without mechanical stress; SH, shade conditions, low water (LW) and mechanical stress (MS). There were five replicate plants for each treatment combination for a total of 60 plants. The shade treatment was created using a cloth which transmits just 10 % of total day light. The low water treatment was established by applying 40 % less water than optimal conditions (60 ml 3x week, see above). The mechanical stress treatment (MS) consisted of gently grasping the plants at the base and flexing them no more than 45^0 for a total of 50 flexures (see further Anten et al., 2005). The MS was done daily during the last 30 days prior to the final harvest.

Soil compaction treatments

The substrate was obtained by a mixture of sand, silt and gravel in a proportion 3:2:1 resulting in a sandy soil texture. This type of soil was chosen as it is most easily compacted. We added a 7 % of NPK fertilizer (7:7:7) to each pot. Different levels of soil compaction were made considering bulk density as reference, i.e., increasing soil mass per same volume unit. No compaction treatment (NC) was made just filling whole volume pot with soil without compacting. For the other two compaction levels, we used an electric hammer (GSH 11 E, Bosch, Germany) with a modified piston to compact. Medium compaction (MC) treatments were made applying the hammer from the top of a soil column (formed by two equal pots, one as soil receptacle and the other as “guide”) of 60 cm in height with an estimated force of 5150 N or 0.52 Tm (percussion frequency 1030 per minute, time of percussion 5 seconds, 15 J per percussion). After that, the guide pot was retired and the spare soil containing in it was discarded having a filled pot. The high compaction (HC) treatment was made using the same procedure but applying an estimated force of 23750 N or 2.42 Tm (percussion frequency 1900 per minute, time of percussion 10 seconds, 15 J per percussion).

In order to describe each treatment of compaction at harvest time, indirect variables were measured: bulk density (dry soil mass between occupied volume in the pot), penetration resistance (by penetrologger, Eijkelcamp, The Netherlands) and soil water content (MWC, Mass Water Content). Soil water content was measured by difference between wet soil sample weight, from each pot, and its dried weight in stove at 105 °C.

Growth and biomass allocation measurements

After 70 days of growth, height of each plant was measured from soil level to the top meristem and the number of leaves was counted. Plants were subsequently harvested destructively and separated into stems, leaves and roots (the latter were carefully washed) of which fresh masses were determined by weighing. Leaf area was measured using a LICOR leaf-area meter (LI3100 LiCor, Lincoln, USA). Stem basal diameter was determined with a caliper. Then stems and leaves were dried at 70 °C for at least three days to obtain leaf and stem dry biomass. Roots were frozen until morphological analysis (see below). Relative growth rate on mass basis was calculated as: $(LnM_2–LnM_1)/(t_2–t_1)$, being M_2 and M_1 the final and initial dry mass of the seedling, respectively and t_2 - t_1 the growth period (70 days) (Hunt, 1990). Initial dry mass was obtained by the product of initial fresh weight and initial dry mass ratio. Dry mass ratio was calculated from a harvest of 10 plants similar to those planted at the beginning of the experiment, calculated as the ratio of dry and fresh mass. Specific leaf area (SLA), biomass fraction dedicated to leaves (LMR, Leaf Mass Ratio), stem (SMR, Stem Mass Ratio) and roots (RMR, Root Mass Ratio) was calculated following Hunt (1990).

Root anatomy

Before roots were dried in stove, a root sample was taken to be used for histological analysis. Approximately 1 cm of primary root from 5 cm to stem-root connection was selected and fixed on FAA solution (acetic formaldehyde: alcohol: acetic acid: distilled water; 10:50:5:35). Then, fixed material was cut by hand with a razor blade raised under binocular magnifying glass Olympus SZ61 (Japan). The slides were made on cross section to longitudinal root axis taking about 100-200 µm in thickness. Cross section samples were stained during 1 minute with astra blue - safranine solution (20:1:0.5, distilled water: astra blue: safranine). Stained samples were placed on a glass slide with water. Images for analysis were photographed using a camera Olympus Altra 20 connected to a microscope Olympus BX41. Xylem cross sectional area (CSA), proportion of xylem CSA dedicated to vessels, mean radius of xylem vessels and xylem vessels frequency (VF) as number of vessels with diameter higher than 10 μm per mm^2 were all measured by means of image analysis software (Image Pro Plus 4.5, Average Cybernetic, Inc., Silver Spring, Md, USA). This criterion avoids confusion with radial cells (in many cases around 10 μm of diameter) and just takes in account those vessels which mainly contribute to total hydraulic conductance.

Root morphology

After defrosting the roots, it was selected for the analysis approximately 66 % of the root excluding first 5 cm, which corresponds with stem-root connection (root base). This was done to avoid an underestimation of specific root length, due that this portion represents low length but with a high biomass. Then, every root sample was scanned to high resolution (600 dpi) by Winrhizo analysis system (Winrhizo ver. 2004a, Regent Instruments Inc., Quebec, Canada). The output gives: mean root diameter, total root length, root volume and length of each diametric class distribution between 0-4.5 mm. The calculated variables were: specific root length (SRL) which is the ratio between root length and its dry mass; total root length as the product of SRL and root biomass (excluding root base); tissue mass density of roots (TMD_R) was calculated as the ratio between root dry mass and root volume; and length of each diametric class. The last was expressed in percentage of total length considering only roots' length of diameter less than 1.5 mm (it means 99 % of total length).

Data analysis

The statistical analysis was made following an ANCOVA analysis considering the 4 treatments as categorical variable (CO, SH, LW, MS) and soil compaction in terms of bulk density as the continuous predictor. A GLM (general linear model) of homogeneity of slopes procedure was made in Statistica 7.1 (StatSoft Inc., Tulsa, OK, USA). Although the compaction treatments were made in a categorical mode, bulk density shows a continuous range, which does not allow us to use it as a categorical factor. In those cases which bulk density showed a significative effect, linear and polynomial regressions were tested using bulk density and bulk density square (second order polinomial) using for that the multiple regression module. The model for each variable's response was selected in function to the highest multiple r coefficient. To test relationships between growth, biomass allocation and root traits, linear correlations were made adjusting p-value with Bonferroni's correction. All statistical analyses were done with Statistica 7.1 (StatSoft Inc., Tulsa, OK, USA).

Results

Growth

Plant biomass, height, dry matter content, RGR were all significantly affected by the different treatments (Table 1). As expected, the highest plant masses were obtained under optimal conditions (control treatment, CO), with an average biomass reduction of -76 %, -53 % and -25 % for shade (SH), low water (LW) and mechanical stress (MS) respectively (Supplementary material, Table S1). For plant height the average reduction relative to the control values was: -30 % (SH), -49 % (LW) and -44 % (MS). Plants grown in SH treatment had the highest percentage of water (94 %) and LW and MS plants had the lowest one (85 % approximately). Relative growth rate (RGR) on a mass basis followed to the same trend already mentioned for biomass; with -29 % (SH), -17 % (LW) and -7 % (MS) compared to the control treatment.

Bulk density had significative effects in all growth variables studied (Table 1), but interestingly, there was a significant interaction the pattern of the response depended on the categorical treatment considered (Table 1, Fig. 1). In the control treatment, responses of height, biomass, dry matter and leaf area to bulk density showed an optimum curve, with values increasing, up to a bulk density of about 1.4 g cm^{-3} and then declining (Fig 1 a-d). In the shade treatment there was no significant effect of bulk density on any of the growth variables (Fig.1 e-h). Finally under low water and mechanical stress all growth values declined linearly with bulk density (Fig.1 i-p).

Table 1. Results of ANCOVA analysis for the different variables studied under different treatments (categorical factor) and bulk density (continuous predictor) and the interaction between treatment and bulk density. The values represent the percentage of explained variance by each factor calculated as (SSx/SStotal) for each model: ns, non significant; a $0.05 < P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. The coefficient sign means the sign of the coefficient of the y variable respect to bulk density in each treatment: CO, control; SH, shade; LW, low water; MS, mechanical stress. For CO, the two signs mean the signs of the two coefficients of a polynomial equation.

		Treatment (CO, SH, LW, MS)	**Bulk density**	**Treatment x Bulk density**	**coeficient sign**			
					CO	**SH**	**LW**	**MS**
	Plant growth and biomass allocation							
B	Biomass (g)	11.50 **	15.11 ***	7.26 *	+-	ns	-	-
H	Height (cm)	6.85 *	16.80 ***	5.22 ns	+-	ns	-	-
DM	Dry Matter (%)	13.20 **	13.53 ***	8.21 *	+-	ns	-	-
RGR_B	Relative Growth Rate on biomass (mg g^{-1} day^{-1})	13.19 **	10.50 ***	9.75 *	+-	ns	-	-
LA	Leaf Area (m^2)	1.11 ns	13.14 ***	0.73 ns	+-	ns	-	-
LMR	Leaf Mass Ratio (kg kg^{-1})	8.45 a	16.68 ***	7.49 ns	+	ns	+	+
SMR	Stem Mass Ratio (kg kg^{-1})	4.80 a	14.56 ***	5.06 a	+-	ns	-	-
RMR	Root Mass Ratio (kg kg^{-1})	17.87 **	5.23 *	14.81 **	ns	ns	+	ns
S/R	Shoot/ Root (kg kg^{-1})	10.95 *	6.32 *	9.43 *	ns	ns	-	ns
SLA	Specific Leaf Area (m^2 Kg^{-1})	26.34 ***	0.22 ns	8.87 *	+-	ns	ns	ns
	Root anatomy and morphology							
XCSA	Xylem CSA (mm^2)	7.16 ns	4.14 ns	6.19 ns	ns	ns	ns	+
RXV	Mean radius of xylem vessels (μm)	2.96 ns	0.46 ns	2.93 ns	ns	ns	ns	ns
PXV_{CSA}	Proportion of xylem CSA dedicated to vessels (%)	6.16 ns	3.10 ns	6.10 ns	ns	ns	-	ns
XVF	Xylem vessels frequency (vessels mm^{-2})	12.70 **	7.31 **	11.83 *	ns	-	ns	ns
RL	Root Length (cm)	21.07 ***	13.57 ***	15.54 ***	-	ns	-	ns
SRL	Specific Root Length (m g^{-1})	10.94 *	3.78 a	8.65 a	ns	ns	-	ns
TMD_R	Tissue mass density of root (g cm^{-3})	26.26 ***	6.71 **	20.57 ***	ns	ns	ns	-
$LDC_{0.5}$	Fine roots (Length diameter class < 0.5 mm) (%)	2.10 ns	6.08 ***	2.76 a	-	ns	-	-
MRD	Mean root diameter (mm)	8.00 ns	9.97 **	10.41 a	ns	ns	ns	+

Biomass allocation

Leaf and stem allocation were not significantly affected by any of the categorical treatments (CO, SH, LW or MS) (Fig. 2), but differences were observed for root mass ratio (RMR), the shoot/root ratio (S/R) and the specific leaf area (SLA, Table 1). Thus, compared to control plants shading caused an 18% reduction in RMR, and a 19% and 180% increase in S/R and SLA, respectively. MS on the other hand increased RMR (+45%) and decreased S/R (-39%) but had no effect on SLA. Finally, low water availability did not induce significant changes in any of these three parameters.

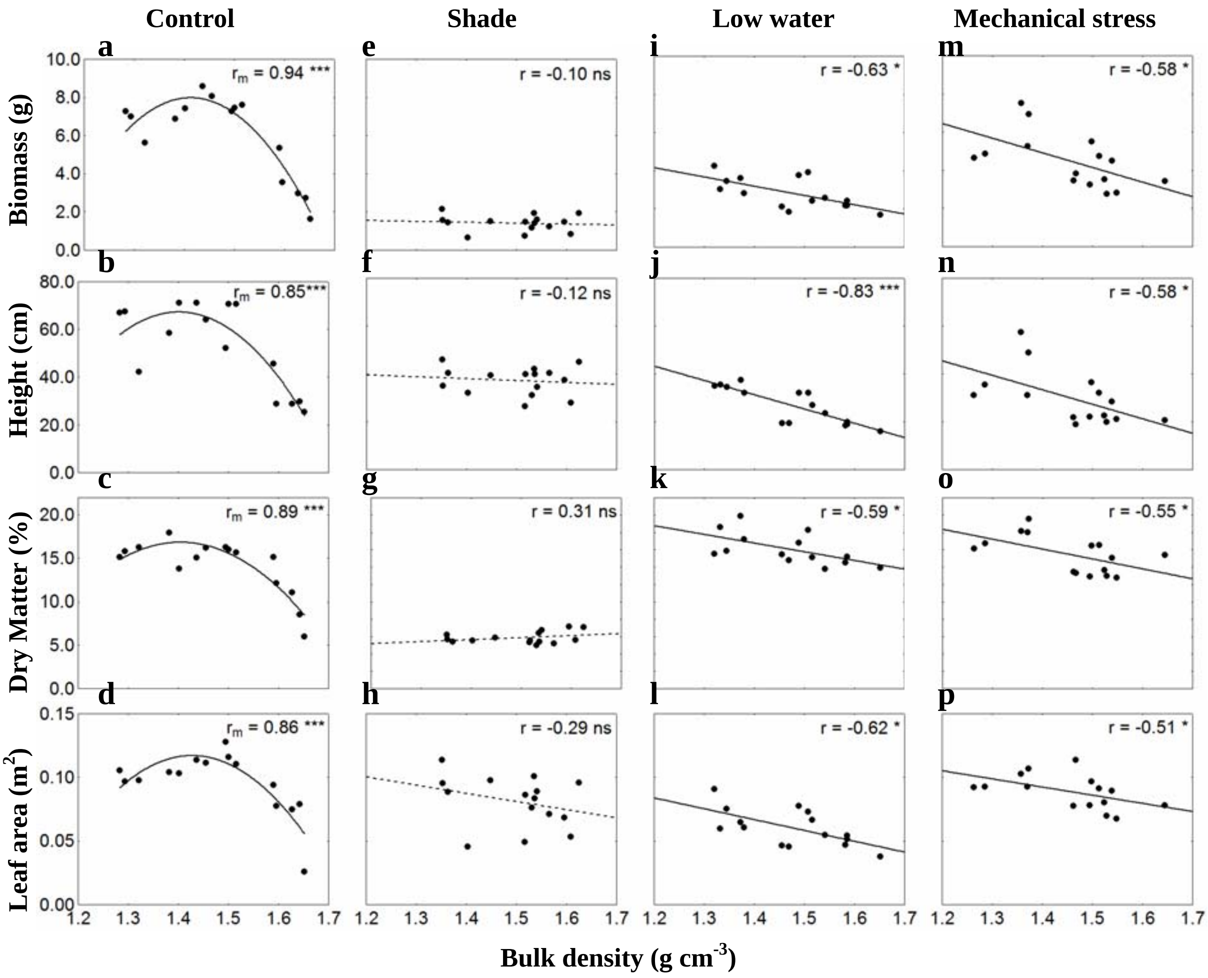


Figure 1. Soil bulk density effects on growth variables of tobacco seedlings under different treatments: control, shade, low water and mechanical stress. Regression coefficients are shown with their signification expressed by: ns, non significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. r_m means multiple regression coefficient for polynomial models.

Regression equations for treatments with significant response were as followed (BD, bulk density; B, biomass; H, height; DM, dry matter; LA, leaf area): Control: $B= -201.0+296.8BD-104.7BD^2$; $H=-1277+1920BD-685BD^2$; $DM=-250.7+381.1BD-135.7BD^2$; $LA=2.35+3.46BD-1.21BD^2$. Low water: $B=9.99-4.85BD$; $H=114.7-59.6BD$; $DM=30.79-10.01BD$; $LA=0.18-0.05BD$. Mechanical stress: $B=15.64-7.66BD$; $H=118.2-60.5BD$; $DM=32.04-11.40BD$; $LA=0.18-0.06BD$.

Soil compaction showed important effects on biomass allocation in all treatments with the exception of shade, which did not show any response to compaction. The leaf mass ratio (LMR) increased while the stem mass ratio (SMR) declined with bulk density (Table 1, Fig. 2). RMR showed a significant increase with bulk density only in the LW treatment. Specific leaf area showed a positive response to bulk density.

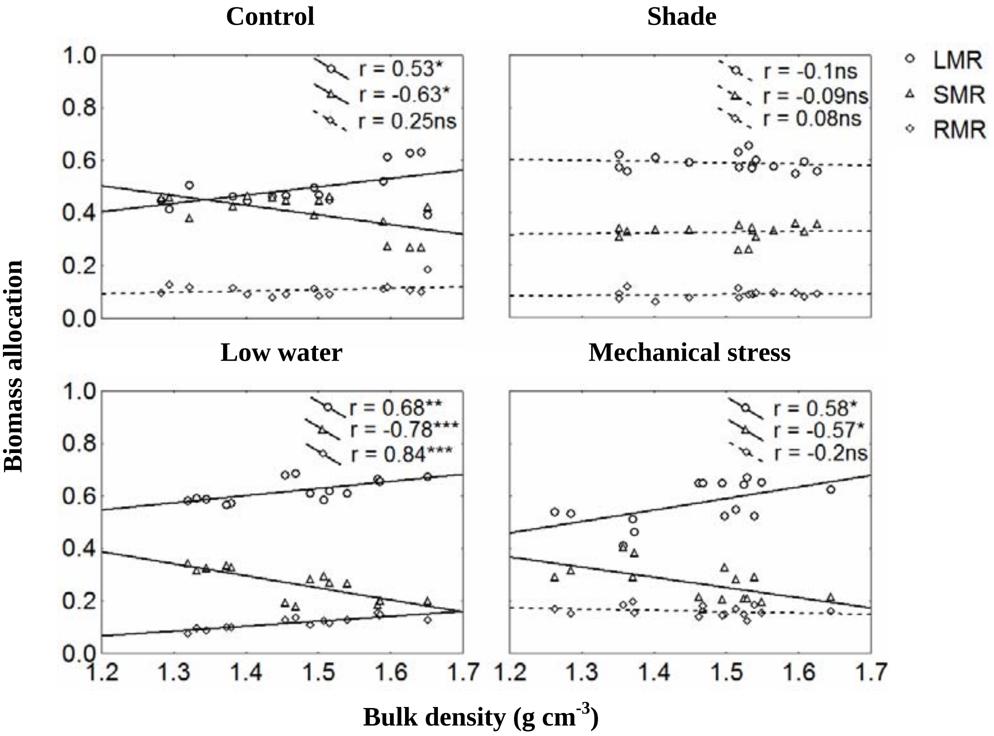


Figure 2. Biomass allocation in relative values for each treatment under soil compaction in terms of bulk density. LMR, leaf mass ratio. SMR, stem mass ratio. RMR, root mass ratio. r, regression coefficient. Signification level is expressed by: ns, non significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Root anatomy

In general, root anatomy variables did not show differences between treatments nor were they strongly affected by soil compaction. Xylem cross section area (XCSA), mean radius of xylem vessels and proportion of XCSA dedicated to vessels seemed not to be affected by the experimental conditions. However, xylem vessels frequency (XVF) presented a significant effect of treatments, bulk density and the interaction T x BD (Table 1). Thus, under mechanical stress XVF was significantly lower than in the other treatments. The effect of bulk density differs between treatments. There was a negative effect of bulk density on XVF In shade, but not in the others treatments (Fig. 3).

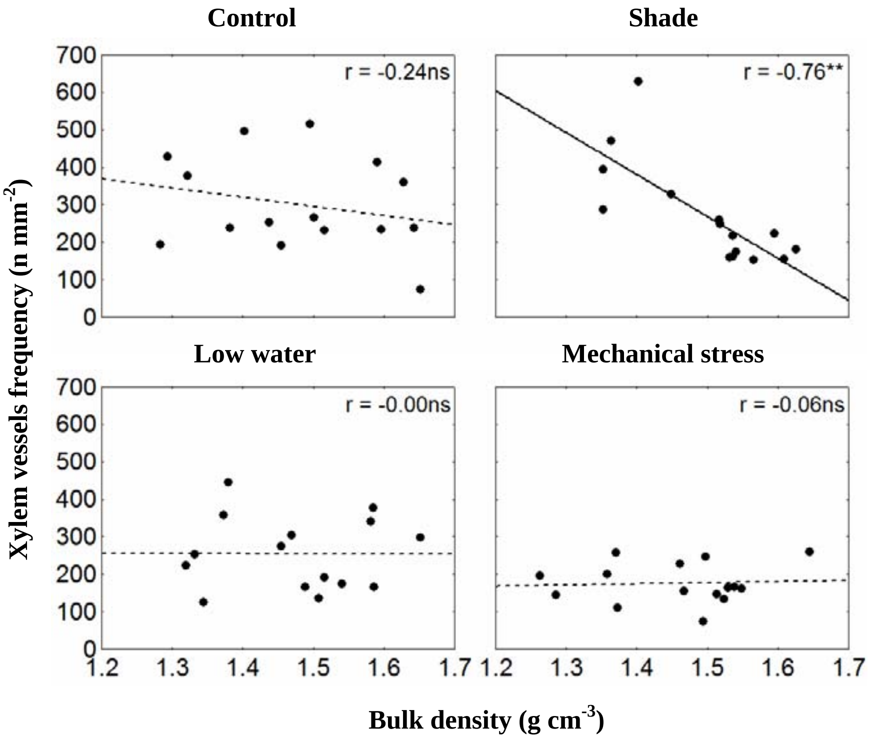


Figure 3. Xylem vessels frequency in response to bulk density under different treatments. r, regression coefficient. Signification level is expressed by: ns, non significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Root morphology

Comparing with control values, root length was strongly reduced under shade (-88 %), low water (-40 %) and mechanical stress (-55 %) (Supplementary index, Table S1). Specific root length showed a strong reduction in plants subjected to mechanical stress (-72 %), which was mostly due to these roots having a higher tissue mass density (+70 %). Under shade treatment plant had a similar effect but in less magnitude. Proportion of fine roots was not affected by treatment, which is reflected on the fact that they had almost the same mean root diameter.

In the control and low water treatments, root length decreased linearly with bulk density (Fig. 4; r = 0.84 and 0.57, respectively). But in the shade and mechanical stress treatments this effect was not observed. Specific root length showed a negative trend with bulk density, which was only significant in the low water treatment (r=-0.59). Tissue mass density linearly decrease with bulk density in the mechanical stress treatment, (r=-0.70), while in the other treatments there was not any significant relationship. The proportion of fine roots and the mean root diameter were negatively affected by bulk density independent of treatment category. In general, soil compaction tends to reduce smaller root diameter classes in favor of larger one.

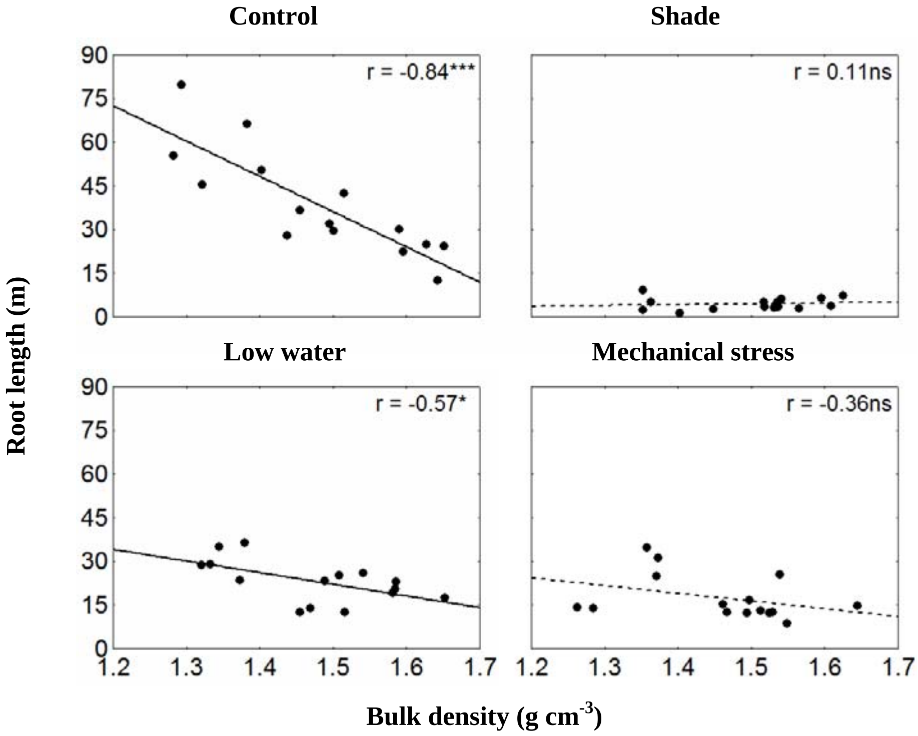


Figure 4. Root length responses to bulk density under control, shade, low water and mechanical stress treatment. r, regression coefficient. Signification level is expressed by: ns, non significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Relationship between plant growth and root traits

Correlations were made without taking in account treatments in order to evaluate the relation degree within plant traits (Table S2). Root length – growth relation appeared as the most conservative trait independently to ambient factors (Supplementary material, Table S2). Biomass, height, dry matter, relative growth rate were correlated with root length, obtaining an average regression coefficient of +0.67. Root length showed relation with biomass allocation as well: leaf area ratio (-0.64), stem mass ratio (0.55) and specific leaf area (-0.63). Specific root length showed a negative correlation with root mass ratio (-0.49), and mean root diameter did it with specific leaf area (-0.48).

Discussion

Soil compaction in control treatment

Our results indicate that under optimal growth conditions (i.e., no limitation of water, nutrient or light and absence of mechanical stress) growth increased with soil compaction (expressed in terms of bulk density) up to a density of about 1.4 g cm^{-3} and then declined. This indicates that at low to intermediate values, soil compaction can stimulate growth. These positive responses have been already reported in literature (Carter, 1990; Håkansson, 1990; Alameda and Villar, 2009). Ardvinsson (1999) explained this positve effect in terms of water and nutrient uptake increment, which was previously shown by Kooistra et al. (1992) as an increase due to a better root–soil contact. Moreover, compaction may increase mass flow transport by increasing the hydraulic conductivity (Kemper et al., 1971). In any case, these effects may change depending of soil texture (Whalley, 2008) and the species studied (Godefroid and Koedam, 2004). On the other hand, negative effects with an increase in bulk density have been explained as the results of root morphology distortion (Chassot and Richner, 2002). Our results show that high compaction levels diminish total root length and the proportion of fine root and that these changes are associated with an increase in mean root diameter. These changes in root morphology seem to be linked to plant growth and architecture. So, negative effects of compaction seem to follow a logical sequence (Alameda and Villar, in prep): root distortion, above growth inhibition, architecture changes and anatomic acclimatization (not found in this study).

Soil compaction under shade

According with our results, plant growth is highly limited under shade conditions (-76 %) and biomass is allocated on very different pattern (specific leaf area 1.8 times greater that control plant). This response to light limitation is well known in literature as shade avoidance which is detected by changes on the ratio of red (R) to far-red (FR) light and translated in hormone response mediated by ethylene and gibberellins (Pierik et al., 2004). The plants growing under shade try to maximize light capture increasing specific leaf area. At the same time above growth is favoured while root growth is diminished which may have important consequences with soil-water interaction. Small and McCarthy (2002) carried out an experiment to simulate disturbances associated to clear-cut harvesting, testing different light and soil compaction treatments. Their results addressed how root growth is generally reduced under shade and it is explained because of reduction of aeration and evapotranspiration rates. In addition, root fineness and density has been reported as shading response by Wahl et al. (2001). If we linked these results with root functioning, we could think that soil conditions do not seem to be relevant to plant growth under shade. This is coherent with our results due there was not any soil compaction effect, despite the exception of a xylem frequency reduction in response at high bulk density. It would be logical to expect limitations imposed by soil compaction at higher growth rates caused by non stable anchorage.

Soil compaction under low water

As expected, low water availability negatively affected growth and height. Interestingly, low water aggravated the negative effects of soil compaction; the relationship between growth and bulk density shifted from an optimum curve to a linearly declining function. The relationship between soil water content and water availability to the plant is strongly determined by physical properties of the soil, and thus also by the degree of compaction. Soil compaction increases the matrix potential of the soil thus decreases water availability. In addition compaction becomes a barrier to increase soil exploration, which was reflected in the reduced root length and specific root length with to soil compaction. As a result plants can explore smaller soil volumes which are particularly detrimental when soil resources are already limiting.

Contrary to our expectations, root anatomy of primary roots did not change under low water regime. An explanation for this result could be that root anatomy changes are mainly produced as result of a water stress situation, different to a low water regime. In the first case, plants have to be able to change some structures to avoid an imminent cavitation process; in the second one, plants adapt previously their growth to water availability. One preliminary conclusion underlines the preserve anatomy traits of tobacco independently to water regime, understood as a low capacity to acclimate their root anatomy. Similarly, Cortina et al. (2008) found small root changes in an experiment with *Pistacia lentiscus* subjected to a contrasting water supply.

Soil compaction under mechanical stress

Shaking treatment produced a slight growth reduction comparing its magnitude with low water and shade treatment. Biomass, height and relative growth rate were lower than control plants; however, leaf area was similar. Biomass allocation was changed in favor to leaf and root investment with a stem reduction. This response can be explained in architecture stability terms. Root anatomy was affected in that direction: a higher xylem cross section area and a lower xylem frequency allow obtaining more solid roots. Scippa et al. (2008) found a lignin increase in roots of plants subjected to mechanical stress. This could be explained by a higher xylem proportion which is based mainly on lignin. Tissue mass density of roots under mechanical stress showed the highest value, accompanied with a specific root length reduction. All these results clearly show the high plasticity of tobacco in response to mechanical stress. Soil compaction under mechanical stress had the same effects already mentioned than control plants, differing just in magnitude. Thus, biomass, height, dry matter, relative growth rate, leaf area and stem mass ratio were linearly reduced with bulk density. The most important difference was found in tissue mass density which is reduced with compaction, together with an increase in mean root diameter.

Relating plant growth and root traits

Root length and plant biomass are related through out a logarithmic function because of the biological implications (Fig. 5). In that sense, the resultant equation seems to present the mean value of samples population. The samples aggregation pattern tends to delimit each treatment in a certain distant to the model. Looking at carefully, it can be seen how this distance reflects the effects of the treatments. Thus, plants subjected to shade showed a narrow linear root length-plant biomass relation, very close to the model. However, the curve seems to delimit two areas: bellow the curve it can be seen plants overestimating root length, which is surprising coherent with low water plants. Above the curve, we can find two groups of plant: first one it is formed close to the model corresponding with plants from mechanical stress; second one it is the most heterogeneous group from control plant. Within it can distinguished points close to the model (corresponding with no compaction treatment), other group far from the curve with a biomass overestimation (moderate compaction) and a third group upon the curve with an underestimation of root length (high compaction). This strong relation plant biomass- root length seems to show the effect of ambient factors in terms of root length ratio terms (Reich et al., 1998).

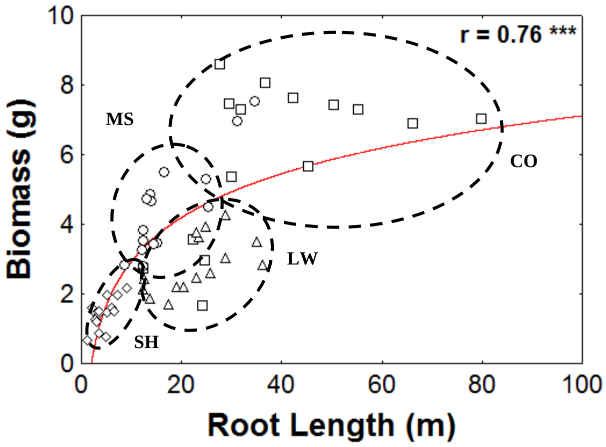


Figure 5. Relationship between plant biomass and root length. A logarithmic regression was performed whose equation is: B = -1.23 + 4.17 log10 RL. Values from each treatment have been remarked to show differences in the followed pattern. CO, control; SH, shade; LW, low water; MS, mechanical stress.

Conclusion

Soil compaction acts on plant development interacting with some abiotic factors. Considering a wide compaction range and optimal conditions of light and water, we would expect to find positive and negative effects on plant growth and a root morphology distortion. In a shade situation, plant growth is directed to maximize light capture, diminishing resources to root growth. That is why soil compaction would need a threshold of root investment to produce changes on above growth under shade. Low water regime limits plant growth and physiology adapting them to water availability. Thus, to maximize water uptake a higher root investment is needed which is impeded by soil compaction; this results in an over-reduction of above growth. Mechanical stress leads changes on plant architecture in order to adapt growth into a more stable structure. Root anchorage is affected by soil compaction in two ways: negatively limiting root growth and positively allowing better stability due to a better soil structure. Therefore, we would expect a trade-off between mechanical stress and soil compaction depending on both intensities.

Acknowledgements

We thank to Elisabeth Giddings for carrying out some stages of the experiment: planting, watering and monitoring. To Fred Siesling and Bas Valstar for their help during the greenhouse work. To Betty Verduyn for helping us with the histology procedure. Esteban Alcántara was essential for the anatomy interpretation. To Simon Cuadros for letting us the morphology analysis equipment. Ronald Pierik provided us tobacco seeds. D. Alameda was financially supported by INTERBOS project (CGL2008-04503-C03-01), Ministry Science and Innovation of Spain.

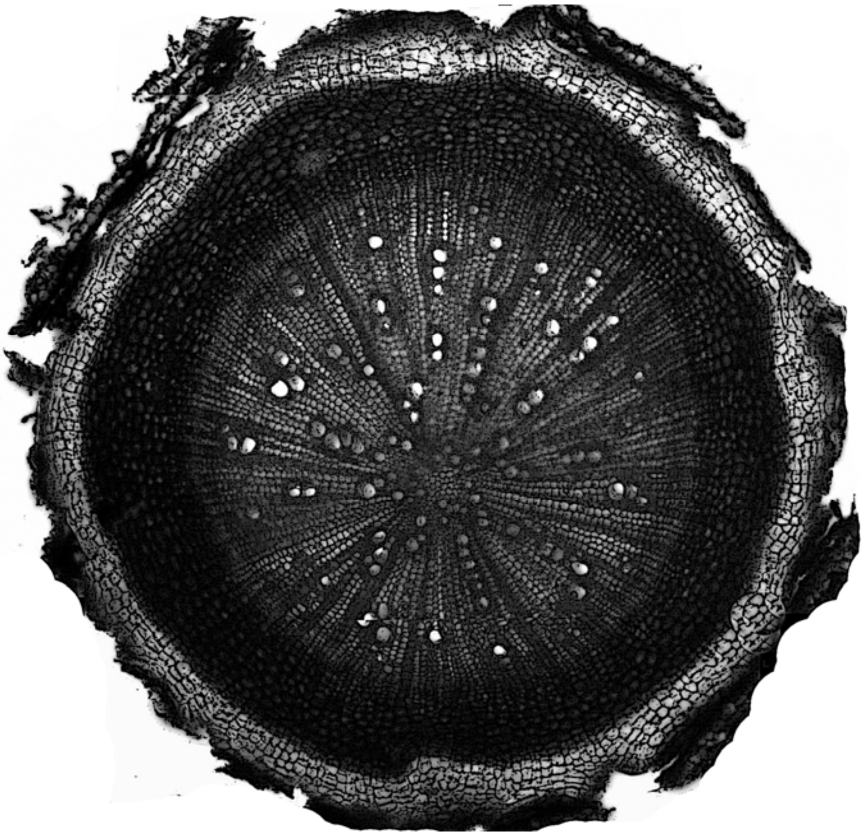
Supplementary index

Table S1. Mean values of growth and biomass allocation for each treatment. RGRb: relative growth rate on biomass basis. SLA: specific leaf area. LMR: leaf mass ratio. SMR: stem mass ratio. RMR: root mass ratio. S/R: shoot-root ratio. LAR: leaf area ratio. XCSA: xylem cross secction area. XV: xylem vessels. XVF: xylem vessels frequency. RL: root length. SRL: specific root length. TMDr: tissue mass density of roots.

	Control	**Shade**	**Low water**	**Mechanical stress**
Biomass (g)	5.96	1.41	2.82	4.47
Height (cm)	52.87	37.2	26.77	29.86
Dry matter (%)	14.06	5.87	16.01	15.42
RGR_b (mg g^{-1} day^{-1})	68.28	48.64	56.9	63.41
Leaf area (m^2)	0.1	0.08	0.06	0.09
LMR	0.49	0.59	0.62	0.57
SMR	0.4	0.32	0.26	0.27
RMR	0.11	0.09	0.12	0.16
S/R	8.7	10.8	7.84	5.3
SLA (m^2 kg^{-1})	34.61	99.9	35.02	36.33
XCSA (mm^2)	0.08	0.09	0.07	0.12
Mean radius XV (μm)	23.37	22.28	20.34	19.91
XCSA dedicated to XV (%)	26.25	32.59	25.7	27.7
XVF (number mm^{-2})	300.44	269.3	255.03	175.82
RL (m)	38.65	4.5	23.05	17.44
SRL (m kg^{-1})	109.39	101.93	110.52	30.81
TMDr (g cm^{-3})	0.1	0.18	0.1	0.33
Fine roots < 0.5 mm (%)	86.36	90.75	83.66	83.4
Mean root diameter (mm)	0.35	0.28	0.38	0.39

Table S2. Correlation matrix for measured variables. Correlations significant at P < 0.001 are shown in bold.

		XCSA	RXV	PXV_{CSA}	XVF	RL	SRL	TMD_R	$LDC_{0.5}$	MRD
B	Biomass (g)	-0.13	0.05	0.00	0.04	**0.76**	-0.13	0.01	-0.19	0.28
H	Height (cm)	-0.21	0.17	0.23	0.21	**0.58**	0.19	-0.17	0.20	-0.11
DM	Dry Matter (%)	-0.16	-0.22	-0.23	-0.04	**0.63**	-0.17	0.04	-0.38	**0.45**
RGR_B	Relative Growth Rate on biomass (mg g^{-1} day^{-1})	-0.12	-0.02	-0.03	-0.02	**0.73**	-0.09	0.00	-0.18	0.30
LA	Leaf Area (m^2)	-0.15	0.12	0.26	0.04	0.32	-0.23	0.15	-0.01	0.10
LMR	Leaf Mass Ratio (kg kg^{-1})	-0.01	-0.13	-0.03	-0.07	**-0.64**	-0.12	0.03	-0.16	0.05
SMR	Stem Mass Ratio (kg kg^{-1})	-0.11	0.19	0.17	0.19	**0.55**	0.31	-0.20	0.28	-0.19
RMR	Root Mass Ratio (kg kg^{-1})	0.29	-0.16	-0.33	-0.31	0.08	**-0.49**	0.42	-0.33	0.34
S/R	Shoot/ Root (kg kg^{-1})	-0.26	0.18	0.37	0.33	-0.15	0.40	-0.31	0.36	-0.37
SLA	Specific Leaf Area (m^2 Kg^{-1})	0.01	0.10	0.34	0.13	**-0.63**	0.16	0.03	**0.44**	**-0.48**



Chapter 6

Linking root traits with plant physiology and growth of *Fraxinus angustifolia* Vahl. seedlings under soil compaction conditions

Submitted to New Phytologist

Linking root traits with plant physiology and growth of *Fraxinus angustifolia* Vahl. seedlings under soil compaction conditions

Abstract

Roots are immersed in a matrix with different and contrasting conditions that may affect its anatomy, structure and function. Soil chemical factors, texture and compaction are some of the main factors that experience roots. This study investigates how soil type and compaction can modify root anatomy and morphology and thus plant growth, architecture and physiology of seedlings of a woody species (*Fraxinus angustifolia* Vahl.). Seedlings were grown in a greenhouse with two types of soil (loam and sandy-loam) under a wide range of soil compaction. Despite intrinsic difference on chemical properties of the two soils, soil compaction showed a great explanatory power of the variation in root morphology and anatomy. Soil type and its chemical composition seem less important than physical properties. Soil compaction affected negatively the proportion of fine roots, specific root length and proportion of root xylem vessels. These effects on root traits had consequences on plant physiology. For example, root tissue mass density was negatively correlated with photosynthesis rate and transpiration rate, and specific root length negatively with leaf water potential. Mean xylem diameter was positively correlated to growth, plant height and plant area. A high root plasticity in different key root traits was found in the same species to overcome soil compaction. In conclusion, soil compaction showed an important effect on root anatomy and structure during the seedling stage, with consequences on plant physiology and growth.

Keywords: bulk density, photosynthesis, relative growth rate, root anatomy, root morphology, soil texture, water potential

Resumen

Las raíces están inmersas en una matriz con diferentes y contrastantes condiciones que pueden afectar su anatomía, estructura y función. Algunos de los principales factores que experimentan las raíces son factores químicos del suelo, la textura y la compactación. Este estudio investiga como el tipo de suelo y la compactación puede modificar la anatomía y la morfología de la raíz y así el crecimiento vegetal, la arquitectura y fisiología de las plántulas de una especie leñosa (*Fraxinus angustifolia* Vahl.). Las plántulas se cultivaron en condiciones de invernadero con dos tipos de suelo (franco y franco-arenoso) bajo un amplio rango de compactación. A pesar de las diferencias intrínsecas en las propiedades químicas de los dos suelos, la compactación del suelo mostró un gran poder explicativo sobre la variación en la morfología y anatomía de la raíz. El tipo de suelo y las propiedades químicas parecen menos importantes que las propiedades físicas. La compactación del suelo afectó negativamente la proporción de raíces finas, la longitud específica de la raíz y la proporción de vasos xilemáticos en la raíz. Estos efectos sobre las características de la raíz tienen consecuencias a nivel de la planta completa. Por ejemplo, la densidad del tejido de la raíz estuvo negativamente relacionada con la tasa de fotosíntesis y transpiración, y la longitud específica de la raíz con el potencial hídrico foliar. El diámetro medio del xilema estuvo positivamente correlacionado con el crecimiento, la altura y el área de la planta. Se encontró una alta plasticidad en diferentes claves características en la misma especie para vencer la compactación del suelo. En conclusión, la compactación del suelo mostró un importante efecto sobre la anatomía y estructura de la raíz durante el estadio de plántula, con consecuencias en la fisiología y crecimiento vegetal.

Palabras clave: *anatomía de raíz, densidad aparente, fotosíntesis, morfología de raíz, potencial hídrico, tasa de crecimiento relativo, textura del suelo*

Introduction

The importance of the hidden half of the plants is one of the subjects of greater interest of contemporary ecophysiology. The doubts about functional aspects of roots are clarified more and more, allowing us to complete the plant physiology knowledge at whole-plant level. Ryser (2006) proposed reasonable doubts about "the mysterious root length", where he showed how root morphology has been investigated intensely from very different fronts, how the ecological conclusions are few and how we continue working without forming a solid theory. At the beginning those hypotheses about the importance and role of root length, root structure, anatomy and development, or about the interaction mechanisms root-soil and root-microorganisms were hardly answered. However, nowadays there is a solid methodology about how to approach the problematic and it seems that we are close to establishing a clear theory.

Hummel *et al.* (2006) have related root structure and anatomy with characteristics concerning whole-plant level in an ample spectrum of grass species. The fundamental conclusion of their study emphasised how independently of species' life-span or botanical families, evidence exists on the strong relation between root structure, morphology and plant physiology traits. Additionally, they observed the necessity to continue the search for root anatomy relations with characteristics of root physiology such as hydraulic conductivity, nutrient and water uptake and root longevity. Rieger and Litvin (1999) found how anatomy root traits were related to hydraulic conductivity, since cortex thickness and presence of a suberised exodermis are the best characteristics to explain hydraulic properties. Wahl and Ryser (2000) took a step further on binding root structure and anatomy with ecological aspects, in such a way that the presence of small xylem vessels and in low number is associated with slow growth strategies and processes of embolism avoidance or resistance against cavitation. Therefore, it seems that a continuity exists in root morphology, anatomy and physiology, which explains traits at whole-plant level as relative growth rate, height or physiology (Wahl and Ryser, 2000).

Plants growing in field conditions experience a wide range of physical soil conditions (Bengough et al. 2006). Under natural conditions it is difficult to separate water limitations effects from other physical factors affecting root growth (Cortina et al. 2008). Soil physical properties such us bulk density, penetration resistance or porosity are descriptors of a complex variable called soil compaction. Soil compaction is a stress factor affecting plant growth mediated by mechanical impedance and/or water and air availability (Bengough and Mullins, 1990; Atwell, 1993). Soil compaction is defined as the increase in the amount of soil particles per volume unit. Different degrees of soil compaction depend on Soil compaction is defined as the increase in the amount of soil particles per volume unit. Different degrees of soil compaction depend on magnitude of disturbance and size/proportion of soil particles. Soil characteristics with great biological importance like air porosity, water capacity or strength are affected by compaction and are dependent on soil type (Whalley et al., 1995). Gomez et al. (2002) showed the influence of soil texture on soil compaction and its interactive effects in biological responses.

In general, it is assumed that compaction makes the following difficult: seedlings establishment due to limitations of radicle penetration, seedlings development due to limitations of root growth and processes mediate by root such as anchorage, water and nutrient uptake, or symbiotic relations. Thus, soil compaction is one of the principal causes of yield crop reduction for a wide species spectrum (Wolkowwsky, 1990; Unger and Kaspar, 1994). In woody plants, it has been shown how soil compaction has many different effects and on different plant traits (Misra and Gibbons, 1996; Kozlowski, 1999). However, these effects vary in function of the species and the compaction range studied. Alameda and Villar (2009) found that low levels of soil compaction are in general positively associated to growth and negatively to root biomass allocation. On the mechanisms of these positive responses, an increase of nutrient transport by diffusion (K and P) and an increase in nutrient transport by mass flow (nitrate and Na) could be implied, determining in both cases a higher mineral nutrition (Arvidsson, 1999) and water absorption (Kooistra, 1992). On the contrary, under high soil compaction, the effects on growth and plant performance are in general negative (Kozlowski, 1999 and 2000).

In the present study, we test the response to compaction of *Fraxinus angustifolia* Vahl. seedlings in two soil types under greenhouse conditions. Variables studied were divided into four categories: root structure (total root length, specific root length, tissue mass density, mean diameter, and diametrical class distributions), root anatomy (xylem proportion and xylem vessels size and number), plant physiology (photosynthesis and transpiration rate, stomatal conductance and leaf water potential) and plant growth and architecture (total biomass, relative growth rate and biomass allocation). The first objective is to know which of these groups of variables are affected and to what degree by soil compaction and/or soil type. The second objective is to relate these variables to explain whole-plant level functioning. Finally, we will summarise all these interrelated effects in a conceptual model, which attempts to explain plant responses to soil compaction.

Materials and methods

Soil types and compaction treatments

The two natural soils used in the study were selected based on: (i) their different texture, allowing to obtain a wide compaction range and (ii) to be representative of Mediterranean ecosystems such as forest soil and a bank (agriculture) soil. Soil type I has a loam texture and was collected along the banks of the Guadalquivir river (“La Golondrina” area, Córdoba, Spain). Soil type II has a sandy-loam texture and comes from a forest of the Sierra Morena mountains (“Los Arenales” area, Córdoba, Spain). Soils were sifted at 1 cm sieve to eliminate stones and vegetal remains. Granulometry and chemical characteristics were completely different (Table 1).

Compaction treatments were carried out at 5.6 and 4.3 % of volumetric water content (FieldScout TDR 100, Spectrum Inc. Technologies) for loam and sandy-loam, respectively. The pots were cylinders of PVC of 50 cm tall and 10.5 cm in diameter (4.33 L), to avoid space limitation for root growth.

Table 1. Mean (± SD) values of physical and chemical characteristics of the two natural soils used in the experiment (n= 30). Significant differences are shown with different letters.

Soil type	I		II	
Textural class	Loam		Sandy-loam	
Gravel < 5 mm > 2 mm (%)	25.5 ± 0	a	60.4 ± 0	b
Sand (%)	46.23 ± 0.38	a	72.36 ± 1.22	b
Silt (%)	36.36 ± 0.38	a	16.43 ± 1.22	b
Clay (%)	17.4 ± 0	a	11.2 ± 0	b
pH 1/2.5	8.24 ± 0.02	a	6.94 ± 0	b
pH en ClK	7.75 ± 0.02	a	6.22 ± 0.03	b
Cation exchange (meq/100)	14.16 ± 0.05	a	14.2 ± 0.27	a
Organic matter (%)	1.07 ± 0.02	a	1.16 ± 0.03	b
N total (%)	0.06 ± 0	a	0.03 ± 0	b
P (Olsen) (p.p.m.)	51.5 ± 0.7	a	3.03 ± 0.19	b
K (p.p.m.)	370 ± 7.07	a	111.67 ± 1.7	b

Three soil compaction treatments: no compaction (NC), moderate (MC) and high compaction (HC) were applied with an identical procedure for both soil types. Five replicates per treatment and soil type were made. The reference to create these treatments was the soil bulk density; therefore by increasing the soil weight for the same volume we increase soil compaction. The procedure for the NC treatments consisted of filling the pot with the aid of other equal pot as a guide up to the total height of 100 cm, after which the column that formed both pots was shaken slightly. For the two remaining treatments we were aided by an electrical hammer (GSH 11 E, Bosch, Germany) with a modified piston to compact (Supplementary Appendix, Fig. S1). MC Treatments were made with the same procedure of NC treatment, and then the hammer was applied up to the height of 75 cm with an estimated force of 5150 N or 0.52 Tm (percussion frequency 1030 per minute, time of percussion 5 seconds, 15 J per percussion). After that, the guide was withdrawn and the spare soil was discarded having the pot full. HC Treatments were made by filling the guide plus the pot only up to the 75 cm approx, height from which the electrical hammer was applied at an estimated force of 23750

N or 2.42 Tm (percussion frequency 1900 per minute, time of percussion 10 seconds, 15 J per percussion). Once an approximate height of 55 cm was attained, the guide was removed where a soil scab was found caused by the hammer percussion. The objective of this methodology was to get a vertical compaction in a natural gradient, as happens in the process of trampling, besides obtaining a repetitive method for treatments and soil types.

Growth conditions and planting

This experiment was developed in a greenhouse at the University of Córdoba (Spain) between July and November 2008. Due to the natural conditions where ash seedlings grow (under adults' canopy in wet habitats where plant cover is high and light availability is low), the experiment was developed under a shadow cloth (transmission of 10% of the total radiation available). Thus the average photosynthetic active radiation (PAR) was 84 ± 13 μmol photon m^{-2} s^{-1} (mean ± standard deviation) (measured with EMS-7, canopy transmission meter, PP-system, UK) during a clear day and the total daily mean radiation throughout the experiment was 2.4 mol m^{-2} day^{-1}. The average temperature of the growth period inside the greenhouse was 17 ± 7 °C. Time of total growth was 124 days. Seeds of *Fraxinus angustifolia* were germinated in trays in a mixture of sand and potting soil in a growth chamber at 25 °C and 60 % of relative humidity. When seedlings had 2 leaves, about 100 seedlings of a similar size were selected. A subsample of 15 seedlings was harvested to obtain the following: fresh and dry biomass of whole plant and per organs (leaves, stems and roots). Dry weight was obtained after at least 2 days in an oven at 70 °C. Each seedling selected for the experiment was freshly weighed and then planted in one pot. The irrigation system was by dripping with a plastic bottle deposit of 0.5 L (model 011, S. A. R. L. IRISO, France). Altogether 6.5 L of water were added to each pot during the first 86 days, after which watering was suppressed forcing the plants to use soil water reserve as a function of soil compaction treatment.

Photosynthesis and water potential measurements

Photosynthesis was measured with a gas-exchange analyser (CIRAS-2, PP System, UK). When leaves did not cover the window of the leaf cuvette, a digital photo was taken to correct the photosynthetic,and transpiration rate and stomatal conductance. The digital photo was analysed with image analysis software (Image Pro Plus 4.5, Average Cybernetic, Inc., Silver Spring, Md, USA). The same leaf used for photosynthesis (using exactly the final foliole from one of the second whorl leaves) was simultaneously used for water potential measurements (Schölander´s chamber, Manofrígido, Lisbon, Portugal). The measurement schedule tried to avoid day-time effects on photosynthesis and water potential; thus in 30 minutes the six different treatments were measured (3 compaction levels x 2 soil types) between 11 a.m. to 2 p.m (solar time). These physiological variables were carried out 20 days after watering was suppressed to determine the plant's ability to use water reserve.

Growth, biomass allocation and soil measurements at final harvest

In order to describe the compaction treatment of each pot at harvest time, bulk density and penetration resistance were measured. When it is necessary to test soil type effects on a compaction experiment, the use of penetration resistance could be confusing due to the inherent water properties of each soil type, which interferes in the penetration resistance measurements (Sojka et al., 2001). Thus, bulk density seems to be a better predictor to test differences due to compaction, independently of soil water content. Therefore, we used soil bulk density to explore the effects of soil compaction. Anyway, penetration resistance was highly correlated with bulk density and results were similar. Penetration resistance was measured by penetrologger (Eijkelcamp, The Netherlands) taking at least twoprofiles for each pot. After the harvest of each plant (see below), the soil mass water content (MVC) was measured in a soil sample for each pot. To do that, one sample for each pot was weighted, and then was dried in a stove to 105 °C for three days. Bulk density was calculated as the ratio between dry soil mass (using the MWC value) and the soil volume occupied in the pot (a cylinder with known radius and height).

Plant height was measured as maximum stem height at harvest time. Each plant was harvested dissecting it in leaves, stems and roots. Roots were cleaned and put in a cool box until their use in anatomic and morphological analysis. Fresh leaves were scanned and leaf area was measured by image analysis software (Image-Pro Plus 4.5). Stem and leaves were dried in a stove at 70 °C for at least 3 days to obtain dry biomass. Relative growth rate (RGR) was calculated following the classical approach (Hunt, 1990) as: $(\mathrm{Ln}M_2–\mathrm{Ln}M_1)/(t_2–t_1)$, being M_2 and M_1 the final and initial dry mass of the seedling, respectively and t_2 - t_1 the growth period (124 days). Initial dry mass for each plant was obtained as the product of initial fresh mass and dry matter content of the first harvest (see above). Specific leaf area (SLA), biomass fraction dedicated to leaves (LMR, Leaf Mass Ratio), stem (SMR, Stem Mass Ratio) and roots (RMR, Root Mass Ratio) were calculated according to Hunt (1990). Leaf area ratio (LAR) was calculated as the product of SLA and LMR.

Nitrogen and carbon concentration of leaves stems and roots were measured in order to evaluate the nutritional state of the plants after the growth period. Dry samples were ground with an electrical mill (IKA, model A10, Germany) and then they were dried again at 70 °C for at least 1 day. Nitrogen and carbon concentration were obtained by an element analyser (Eurovector EA 3000; EuroVector SpA, Milan, Italy).

Root morphology

A fraction of root biomass was taken to analyse the morphological traits of each plant. This fraction represented about 20 ± 5 % of total root biomass (mean ± SD), and in morphological terms this fraction represents one of the main roots (first order) linked to the root-stem connection. This approach was thought to analyse functional root length, avoiding underestimation caused by root base, which represents high biomass and little length. Roots were scanned with a high resolution scanner at 600 dpi in a transparent tray with water. Root analysis was carried out with image analysis software (WhinRHIZO ver. 2004a, Regent Instruments Inc., Quebec, Canada). The output of the software gives the following: mean root diameter, total root length, root volume and length of each diametric class distribution between 0-4.5 mm. After the scanning, roots were dried at 70 °C to obtain dry biomass. The calculated variables were as follow: specific root length (SRL) as a ratio of root length and dry mass; total root length as a product of SRL and dry mass of the whole root (excluding root base); root tissue mass density (TMD_R) was calculated as a ratio of root dry mass and root volume; root length ratio (RLR) as a ratio of total root length and plant mass. Length of each diametric class was expressed in a percentage of total length considering only the roots' length of diameter less than 1.5 mm (which represents 98 % of total length).

Root anatomy

Root samples for anatomy were taken using the same criterion for all samples: we selected 2 cm of main root or one of the greater diameters (in case unique main root does not exist) at 5 cm approximately from stem-root connection. Samples were fixed with FAA (acetic formaldehyde: alcohol: acetic acid: distilled water; 10:50:5:35) until its processing in laboratory. Fixed material was cut by hand with shave blades raised under an Olympus SZ61 binocular magnifying glass. The slides were made on a cross section to longitudinal root axis taking about 100-200 μm in thickness. Cross section samples were stained for 1 minute with toluidine blue solution (0.5 % in distilled water), which stains lignin blue and cellulose purple (Feder & O'Brien, 1968), then they were washed in water to eliminate colorant excess. Stained samples were placed on a glass slide with water. Images for analysis were photographed using an Olympus Altra 20 camera connected to an Olympus BX41 microscope. Measurements were made over a subsection along a symmetry axis, thus relative values are referred to the surface measured (50%). The percentage of xylem per cross section area (CSA) was calculated, and the number and proportion of xylem vessels over total xylem CSA and mean diameter of xylem vessels.

Efficiency and susceptibility to damage during water conduction was evaluated through relative hydraulic conductivity (Zimmermann 1983). The relative hydraulic conductivity was estimated by using a modified Hagen–Poiseuille equation (Fahn et al., 1986): RC = r^4VF, where RC is the relative hydraulic conductivity, r the mean vessel radius and VF the vessel frequency.

Statistical analysis

The statistical analysis was made using ANCOVA analysis considering soil type (categorical variable) and soil compaction in terms of bulk density (continuous variable) with the GLM of homogeneity of slopes procedure in Statistica 7.1 (StatSoft Inc., Tulsa, OK, USA). According to the results in Table 2 we could not perform ANOVA or factorial ANOVA in two ways (with soil types and compaction treatments as factors) due to the different response of each soil to the same perturbation system. For instance, high compaction level to loam soil is comparable to low compaction level of sandy-loam soil. So, compaction was considered a continuum variable using bulk density data instead of a categorical factor with three levels. Linear correlations were made to find out the relationships between the variables.

Results

The two natural soils showed very different properties besides the physical one (Table 1). Soil texture, nutrient concentrations, pH, moisture, bulk density and penetration resistance draw two very different environment. Thus, a plant grown in a loam soil (type I) would have more nutrients (N, P, K) and a lower compaction than those in sandy-loam soil. These contrasting differences in chemical and physical conditions, together with their response to compaction, may affect many biological processes at the same time. In the following sections we will present the effect of soil compaction and soil type on different variables of plant performance. In general, we found that soil compaction strongly affects root anatomy and structure which has consequences on plant physiology. However, soil type did not have any strong effect on those variables.

Root morphology

Mean root diameter, root length, specific root length (SRL) and diameter classes' distribution were strongly influenced by soil bulk density but soil type did not have any significant effect (Table 2). SRL was negatively affected by bulk density (Fig. 1a), showing a 64 % reduction with a 25 % bulk density increment. Length of different root orders was affected by compaction. Depending on which class diameter was considered, bulk density had a different effect: fine roots (diameter between 0 – 0.5 mm) were negatively affected by bulk density and those of medium and high diameter (between 0.5 – 1.5 mm) were positively affected (Fig. 1b). These changes in root size class distribution with compaction were visually evident (Fig. 2), where a second and third root orders reduction can be seen at the same time as first root order was incremented and its diameter was thicker. Despite these changes on the general root morphology pattern, root tissue mass density (TMD$_R$) was not affected by soil type or compaction.

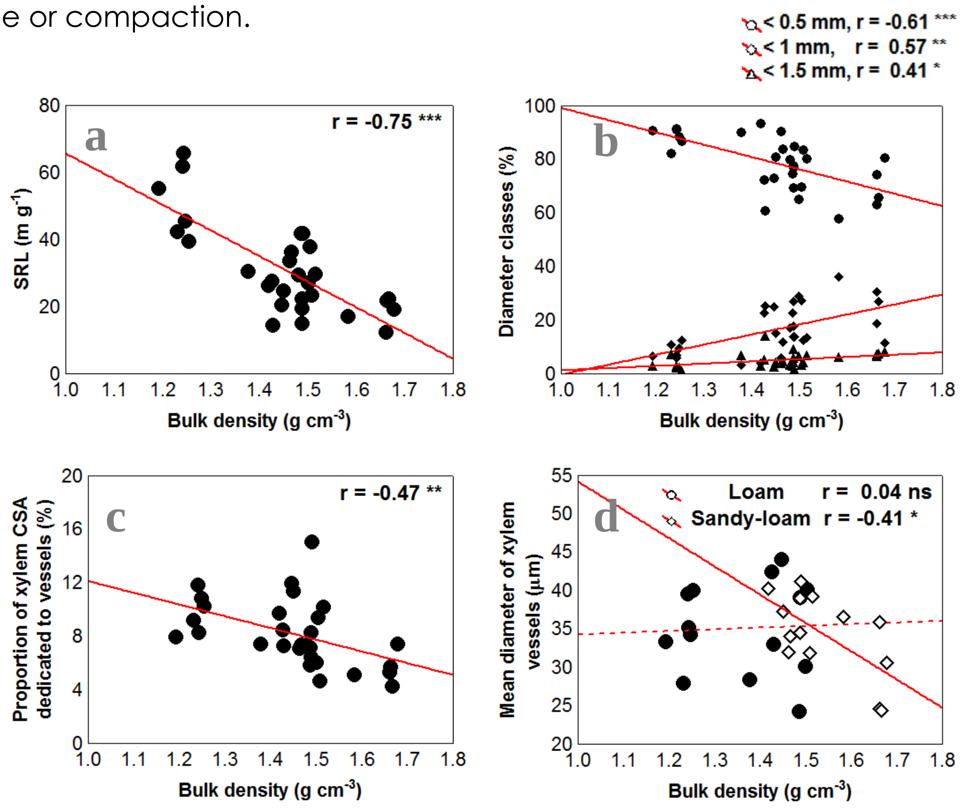


Figure 1. Bulk density relationship with (a) specific root length (SRL), (b) diameter classes of roots, (c) proportion of xylem dedicated to vessels, and (d) mean diameter of xylem vessels.

Table 2. Results of ANCOVA analysis for different variables studied using soil type as categorical factor and bulk density as a continuous predictor. The values represent the percentage of explained variance for each factor and the interaction. ns, non significant; a 0.05 < P < 0.10; * P < 0.05; ** P < 0.01; *** P < 0.001. Below the column r sign is shown the correlation sign of the variable studied against bulk density in each soil type (I and II) and considering all data together.

		Soil type (I and II)	Bulk density (g cm^{-3})	Soil type x Bulk density	r sign		
					I	II	all
	Root morphology						
MRD	Mean root diameter (mm)	0.51 ns	51.21 ***	0.11 ns	+	+	+
SRL	Specific Root Length (m g^{-1})	2.45 ns	23.68 ***	2.52 ns	-	-	-
RL	Root Length (cm)	1.29 ns	23.53 ***	1.23 ns	-	-	-
TMD$_R$	Tissue mass density of root (g cm^{-3})	6.15 ns	5.44 ns	3.79 ns	ns	ns	ns
LDC$_{0.5}$	Length of diametric class > 0 < 0.5 mm (%)	0.03 ns	22.04 ***	0.01 ns	-	-	-
LDC1$_{.0}$	Length of diametric class > 0.5 < 1 mm (%)	0.02 ns	40.45 ***	0.29 ns	+	+	+
LDC1$_{.5}$	Length of diametric class > 1 < 1.5 mm (%)	2.19 ns	16.13 *	1.89 ns	+	+	+
	Root anatomy						
X$_{CSA}$	Xylem CSA (%)	0.00 ns	0.10 ns	0.01 ns	ns	ns	ns
PXV$_{CSA}$	Proportion of xylem CSA dedicated to vessels (%)	4.13 ns	15.12 **	3.54 ns	-	-	-
NXV$_{CSA}$	Number of xylem vessels per mm^2 of xylem CSA	3.57 ns	2.07 ns	3.47 ns	ns	ns	ns
DXV$_{CSA}$	Mean diameter of xylem vessels (µm)	7.56 a	5.69 a	7.25 a	ns	-	ns
RC	Relative hydraulic conductivity (µm^{4}10^6)	6.67 ns	10.00 a	6.34 ns	ns	-	ns
	Plant growth and architecture						
H	Height (cm)	7.81 ns	0.90 ns	8.93 a	ns	-	ns
B	Biomass (g)	5.05 ns	0.12 ns	5.99 ns	ns	ns	ns
RGR	Relative Growth Rate (mg g^{-1} day^{-1})	10.52 a	0.02 ns	12.54 *	ns	ns	ns
LA	Total Leaf Area (cm^2)	17.79 *	0.35 ns	19.16 **	ns	-	ns
SLA	Specific Leaf Area (m^2 Kg^{-1})	17.28 **	0.65 ns	14.33 **	+	-	+
LMR	Leaf Mass Ratio (kg kg^{-1})	22.17 **	0.82 ns	23.71 **	+	-	ns
SMR	Stem Mass Ratio (kg kg^{-1})	0.14 ns	1.45 ns	0.34 ns	ns	ns	ns
RMR	Root Mass Ratio (kg kg^{-1})	13.64 *	1.53 ns	15.31 *	ns	+	ns
LAR	Leaf Area Ratio (m^2 Kg^{-1})	28.44 ***	1.33 ns	28.39 ***	+	-	ns
RLR	Root Lenght Ratio (root length/plant mass) (m g^{-1})	6.27 *	21.39 ***	6.38 *	-	ns	-
	Plant physiology						
Pn	Net photosynthesis rate (µmol CO$_2$ m^{-2} s^{-1})	1.88 ns	0.42 ns	3.07 ns	ns	ns	ns
E	Transpiration rate (µmol H$_2$O m^{-2} s^{-1})	0.35 ns	0.82 ns	0.19 ns	ns	ns	ns
Gs	Stomatal conductance (µmol H$_2$O m^{-2} s^{-1})	1.28 ns	1.08 ns	0.90 ns	ns	ns	ns
Ci	Internal concentration of CO$_2$ (ppm)	7.22 a	0.00 ns	8.46 a	ns	ns	ns
Ψ_L	Leaf water potencial (Bar)	0.76 ns	21.04 *	1.32 ns	-	ns	-

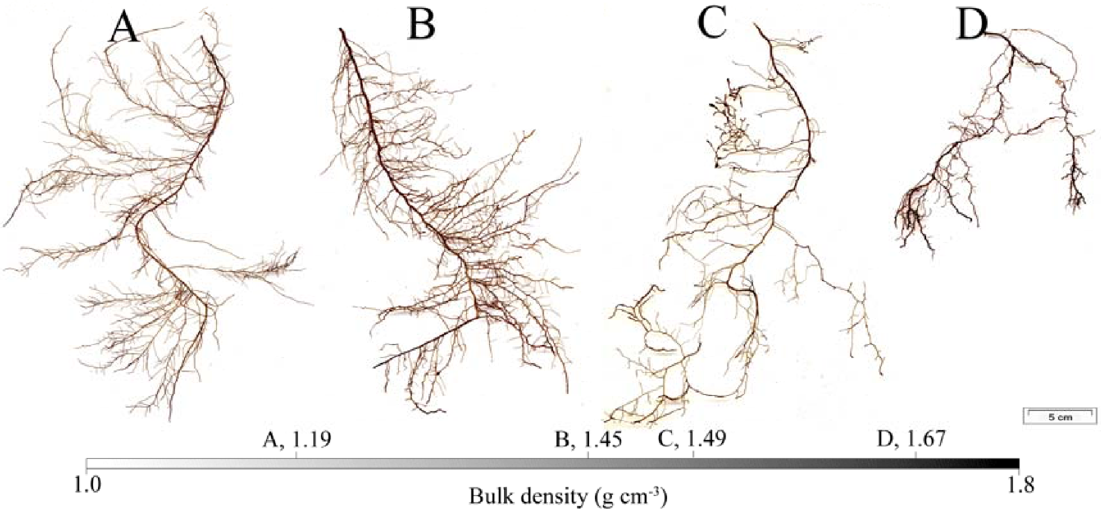


Figure 2. Pictures of root morphology of *Fraxinus angustfolia* seedlings under different soil treatments. A, no compaction of loam soil; B, high compaction of loam soil; C, no compaction of sandy-loam soil; D, high compaction of sandy-loam soil.

Root anatomy

Proportion of xylem was not affected by bulk density or soil type (Table 2). Proportion of xylem cross section area (CSA) dedicated to vessels, which it is related to percentage area for water conduction, was negatively affected by bulk density (Fig. 1c) and not by soil type (Table 2). Number of xylem vessels per unit area of xylem CSA was not affected by bulk density or soil type, however, xylem vessels diameter only was negatively affected by soil compaction on sandy-loam soil (Table 2, Fig. 1d). Relative hydraulic conductivity was slightly affected by bulk density on sandy-loam soil, indicating that high compaction conditions produces a loss in hydraulic conductivity as results of diameter decrease. In Figure 3 can be seen the different tissues measured to the analysis.

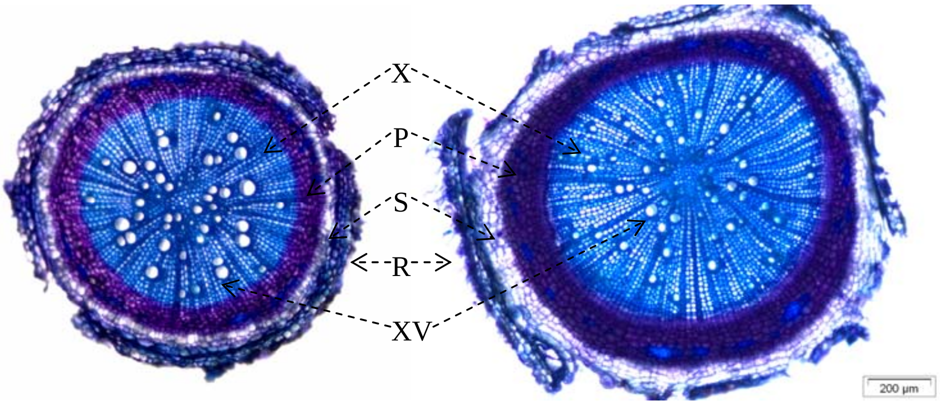


Figure 3. Pictures of root histology of *Fraxinus angustifolia* seedling under compaction treatments: no compaction treatment of loam soil is shown on the left; high compaction treatment of sandy-loam soil on the right. X, xylem; P, phloem; S, suber; R, rhizodermis; XV, xylem vessels.

Plant growth and architecture

Growth variables such as plant height and total biomass were not modified by soil type, nor by soil compaction (Table 2). In general, most of the growth and morphology variables showed a significant interaction between soil type and soil compaction, which means that the soil compaction effect varies depending on soil type. Specific leaf area, leaf mass ratio and leaf area ratio showed an increase with bulk density in the richer soil (loam soil, type I) (Table 2). However, the same variables showed a decrease with soil compaction in the poorer soil (sandy-loam soil, type II). Root mass ratio was affected by soil type with a significant interaction with bulk density due to a different response within the soil type. For sandy-loam soil bulk density produced a significant increase in root mass ratio whereas in loam soil there was no effect of bulk density (Table 2). The relation between root length and plant biomass (root length ratio, RLR) was negatively affected by bulk density but also by soil type with a significant interaction between both variables. This is because in loam soil RLR was negatively affected by compaction while on sandy-loam it was not significantly affected (Table 2).

Chemical composition of some tissues was also affected by soil compaction. Leaf N concentration was slightly incremented with bulk density in loam soil (r=0.50; *P*=0.06), and root C was negatively affected by bulk density in loam soil (r=-0.65; *P*=0.01).

Plant physiology

Photosynthesis and transpiration rate, stomatal conductance and internal CO_2 concentration did not show significant differences between soil types and soil bulk density (Table 2). However, leaf water potential was affected by bulk density, resulting in higher water stress with higher soil compaction conditions (Table 2, Fig. 4).

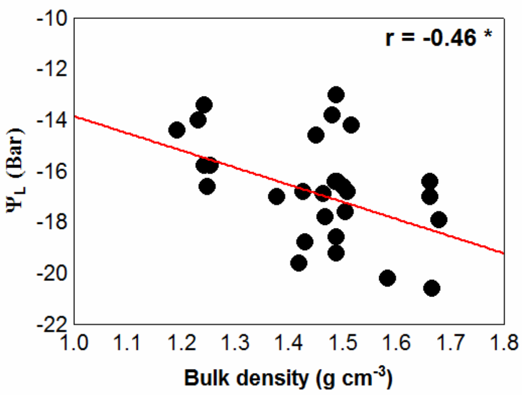


Figure 4. Relationship between leaf water potential from plants of both soil type and bulk density.

Whole plant functioning

The four groups of variables considered (root structure, root anatomy, plant growth and architecture, and plant physiology) showed different relationships with each other (Table S2). First, root morphology was correlated mostly with physiology. For example, root tissue mass density was negatively related with photosynthesis (Fig. 5a), transpiration rate and stomatal conductance, whereas specific root length was positively related to the same variables. Besides, SRL had a significant positive effect on leaf water potential (Fig. 5b). On the contrary, root morphology has just a few significant effects on growth and architecture variables; i.e. root length was positively related to total biomass (Fig. 5c) and height, and negatively with specific leaf area (SLA).

Secondly, root anatomy showed relationships mainly with growth and architecture variables. For example, mean diameter of xylem vessels CSA seems to be closely associated with growth, i.e., height, biomass, leaf area and leaf mass ratio were positively associated with a mean diameter increase (Fig. 5d), whereas root mass ratio did it negatively.

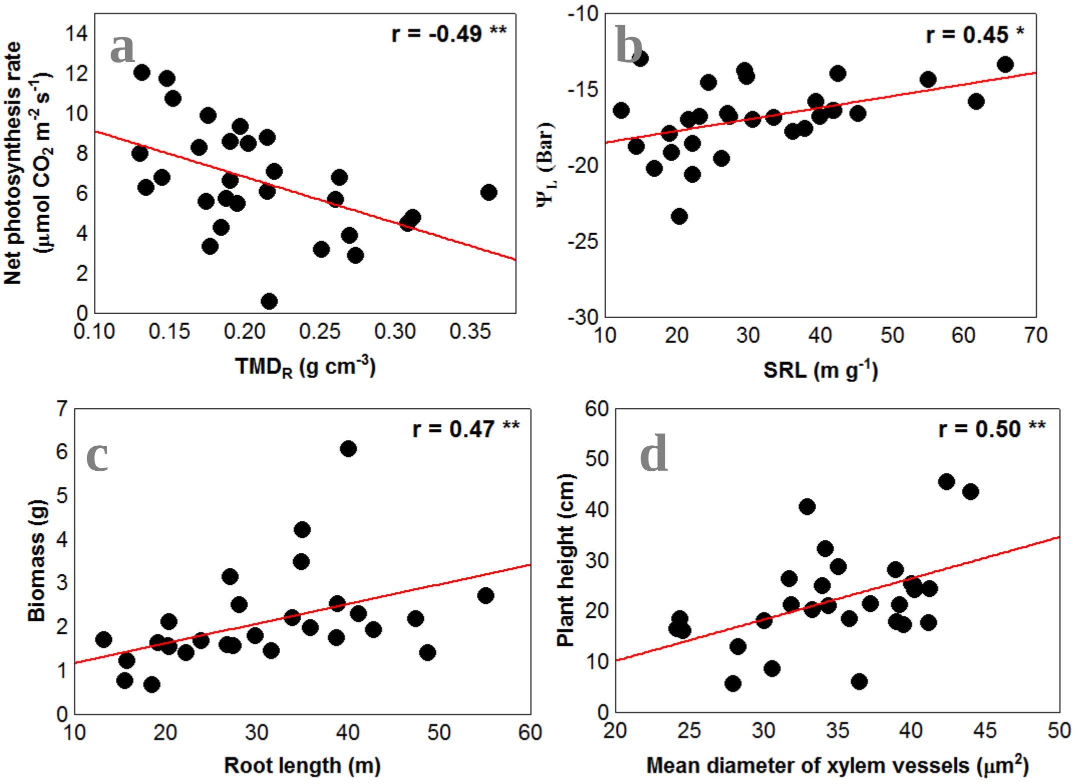


Figure 5. Regression between studied variables to explain functioning at whole-plant level.

Discussion

Soil type response to compaction

It has been found that soil compaction effects on soil physical and mechanicals properties depend on soil texture (Unger and Kaspar, 1994). So, although the increase rate of bulk density due to compaction between two soil types was not very different (+20 % for loam and +13 % for sandy-loam), the sandy-loam soil showed a higher bulk density in absolute values, due to its particles density. It is noticeable that compaction treatment was carried out at low water content and approximately the same for both textures to discard water effects. Our results showed that it would be needed to apply more energy to a loam soil to raise bulk density values. Therefore, soil disturbances can increase compaction according to soil texture, with larger granulometries being more susceptible to compaction. This interrelation between compaction and texture has been used in order to establish susceptibility classification in compaction hazard as a function of soil texture (Nhantumbo, 2006). Although compaction treatments did not enhance water retention, loam soil showed having better structure to maintain water reserve. Whalley et al. (2008) studied different properties of two types of soil (loam and sandy) under compaction. They found how loam soils could benefit from a compaction disturbance because of a bimodal porosity is created, allowing roots to grow in macropores and obtaining higher water retention (micropores) than sandy soil.

In relation to chemical properties of soil under compaction, it seems to be logical that soil compaction increases nutrient concentration per unit volume. Taking into account chemical differences between loam and sandy soil, we would expect plant nutrition to be affected. We found that leaf N concentration was slightly incremented with compaction in loam soil, which could be due to a better root-soil contact which allows higher nutrient transport rates (Arvidsson, 1999). Root C was negatively affected by compaction in loam soil, which could be explained by root morphology changes induced by compaction: higher diametric classes are related with secondary root growth which increases xylem growth and hence lignin (C) concentration.

Linking root traits with plant physiology and growth

Our main objective with this work was to extract a general idea about how soil type and soil compaction affects different root traits and how these traits can be linked to plant functioning. Soil compaction had a negative effect over root elongation, which is translated into a root length decrease, a root diameter increase and changes on root classes' diameter distribution. Whalley et al. (1995) and Mosena and Dillenburg (2004) have found similar results, with shorter and thicker roots in highly compacted soils. The decrease in specific root length (SRL) with soil compaction is also commonly reported in literature (Bengough and Mullins, 1990; Watt et al., 2005). Atwell (1993) explained this change as a mechanical impedance effect, because longitudinal growth is reduced in favour of a radial one. Cellular implications were addressed by Dexter (1987) as a reduction of root cell elongation and production rate accompanied by a radial cell extension. It has been suggested ABA and ethylene's activity leading this signal caused by compaction (Hussain et al., 2000; Roberts et al., 2002; Whalley et al., 2006). Considering the whole root system, compaction decreased second and greater root order's growth accompanied by an increase of first root order's growth. Subsequently, when normal development of roots system is impeded, processes such as anchorage, water absorption and nutrient uptake can be affected. In that sense, difficulties have been reported in isolating soil compaction stress from water stress (Taylor and Ratlif, 1999; Whalley et al., 2007b). Soil compaction and water stress can be considered cause-and-effect, due to the first one reducing exploring capacity of roots, resulting in lower water uptake. Moreover, soil compaction increased soil matric potential (Taylor and Ratliff, 1969), which makes it difficult to extract water from the soil. In our study, leaf water potential, as a water stress indicator, was negatively correlated with bulk density, which means higher water stress (more negative values) with higher soil compaction. The same results were found by Liang et al, (1996). On the other hand, specific root length (SRL) was positively correlated to leaf water potential, indicating that larger and thinner roots can reduce probabilities to reach a water stress situation. Similarly, SRL was positively correlated with transpiration rate and stomatal conductance, which could mean that evaporative demands were not interrupted thanks to the uptake throughout thinner roots. Hund et al. (2009) found a root diameter reduction under water stress conditions, which is explained by a restriction of lateral expansion of root stele and cortex in the apical zone (Liang et al., 1997). This result would be coherent with Steudle (2000), who describes in "a composite transport model" which root hydraulic variables can explain the ability of roots to take up water, and which factors affect water uptake. Thus, a root cortex width could be understood as a limitation to water uptake from soil to xylem vessels due to its hydraulic resistance. In our work, root anatomy changes were due to soil compaction but no effect of soil type was found. For instance, proportion of xylem cross section area dedicated to vessels was negatively related to bulk density, and mean diameter xylem vessels decreased with bulk density (only in sandy-loam soil). These changes can be explained as a response to a water deficit situation (Holste et al., 2006).

Finally, we should be able to link growth variables to root traits. It seems to be difficult assuming that soil compaction did not affect growth by itself, which could be explained by the light conditions in the experiment (10 % of total daylight). Light must be taken in account as a filter of soil compaction effects, due to soil compaction starting to be relevant at a specific light threshold (Small and McCarthy, 2002; Alameda et al., in preparation). However, bulk density showed important effects, and in opposite directions, on plant architecture depending on which soil type is considered. This different effect of soil compaction on plant architecture depends on soil texture and water regime and it may be explained as a result of the root architecture changes already mentioned. In any case, growth and architecture showed relations with root traits. Root length was positively related with biomass and height. Also, mean diameter of xylem vessels was positively correlated with biomass, height, leaf area and leaf mass ratio. According to Wahl and Ryser (2000) and Hummel et al. (2006), mean diameter of xylem vessels is closely related to height on a wide spectrum of herbaceous species, which can be associated to an important aspect of the trade-offs between anatomical structures, hydraulic conductance and plant height which determine plant characteristics at the whole-plant level and the ecological behavior of a species.

To sum up, we built a conceptual model attempting to describe causal relations. The objective is to understand the effects of soil compaction from soil to whole plant functioning (Fig. 6). It seems logical to find out an effect at different scales, i.e., soil bulk density modifies root morphology which is translated in a water stress situation and this produces changes on root anatomy as a result of an acclimatization process. It would be necessary to study in depth the causal relations connecting below and above changes induced by soil bulk density.

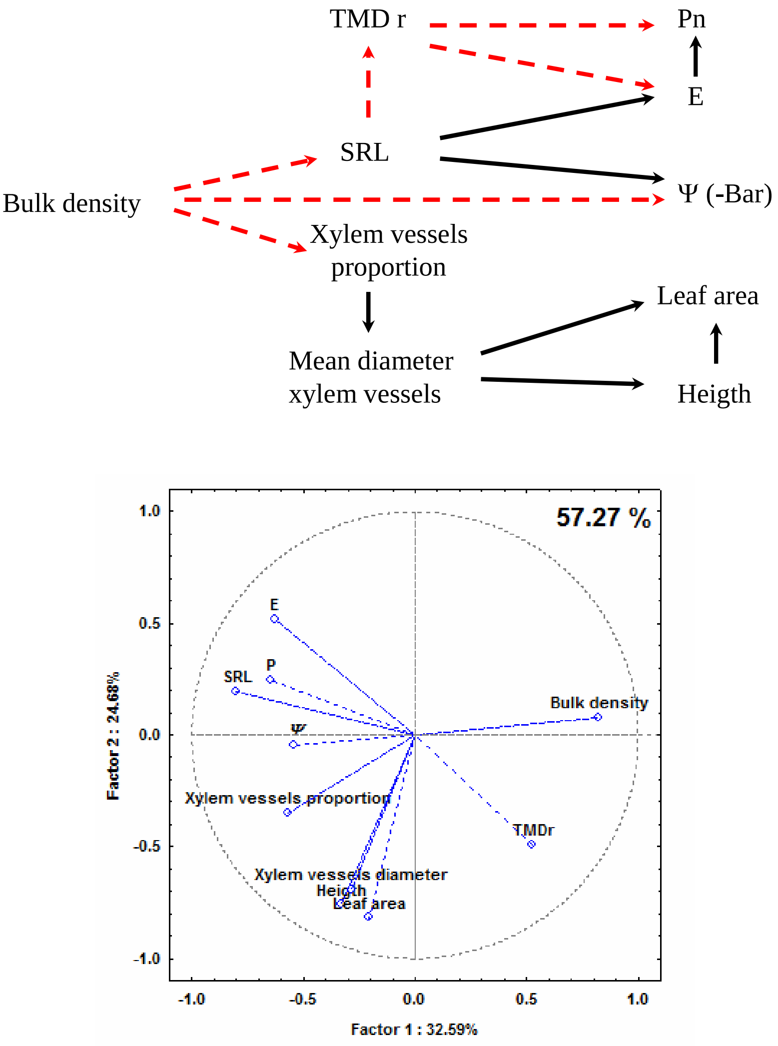


Figure 6. Conceptual model and PCA analysis. The variables presented in the model are matched by arrows attempting to draw negative (dash) and positive (solid) causal relations. PCA analysis is shown as a statistical tool to support the model based on correlations. The PCA explains 57.27 % of the variantion in these variables. TMDr, tissue mass density of roots; SRL, specific root length; Pn, photosynthesis rate; E, evapotranspiration rate; Ψ, leaf water potential.

Interestingly, we found that *Fraxinus angustifolia* is most sensitive to soil physical properties instead of chemical ones. Bejan (2008) regards the plant as a physical flow architecture that evolves to meet two objectives: maximum mechanical strength against the wind, and maximum access for the water flowing through the plant, from the ground to the atmosphere. Thus, the first stages of development of trees need to reach a place where they anchor themselves in a minimum of light, water and nutrient requirements. Therefore, soil physical properties may act as a first filter on the establishment, giving shape to the future juvenile.

Conclusions

Bulk density modifies root morphology as a result of mechanical impedance. This modification is translated to changes on physiology, growth and architecture at whole-plant level. Root anatomy plasticity seems to allow certain acclimatization to a water stress situation, changing in order to maintain hydraulic conductivity within the limits imposed by water availability in compacted soils. *Fraxinus angustifolia* appears to be more sensitive to soil physical properties instead of a chemical one during the seedling stage. It would be necessary to know if this is a general response on woody plants and therefore more studies including a wide set of species are required.

Acknowledgments

We are grateful for the help received from many colleagues at different stages of the experiment and wish to thank the following: Manolo Olmo, Elena Suárez, Pepón Vacas, Alejandro Mesa, Elisabeth Giddings, Paco Conde, David Gómez, Cristina Gómez, Joaquín Reyes, Puri Cabello, Simón Cuadros, José Luis Ubera, Esteban Alcántara and Niels Anten. This experiment was funded by the DINAMED (CGL2005-05830-C03-2/BOS) and INTERBOS (CGL2008-04503-CO3-02) projects funded by the Ministerio de Ciencia e Innovación and FEDER funds. This coordinated research line forms part of the GLOBIMED National Thematic Network (http://www.globimed.net/).

Supplementary index

Table S1. Properties of two soils used in the experiment under different compaction treatments. Factorial ANOVA was made to evaluate significative differences (shown with different letter).

Soil type	N	Compaction treatment	Bulk density (g cm-3)	Penetration resistance (MPa)	Mass Water Content (%)
Loam (I)	5	1	1.23 ± 0.02 a	0.65 ± 0.43 a	12.63 ± 3.49 a
	5	2	1.37 ± 0.08 b	1.98 ± 0.62 b	10.81 ± 2.87 a
	5	3	1.49 ± 0.02 c	3.00 ± 1.44 c	10.11 ± 3.41 a
Sandy-loam (II)	5	1	1.46 ± 0.03 c	3.32 ± 0.23 c	3.85 ± 0.63 b
	5	2	1.50 ± 0.01 c	2.48 ± 0.67 bc	5.28 ± 0.69 b
	5	3	1.65 ± 0.04 d	4.32 ± 0.21 d	5.61 ± 1.30 b

Figure S1. Model system to compact soils.

Table S2. Correlation matrix for measured variables. Correlations significant (Bonferronni’s correction) at P < 0.0002 are shown in bold.

			SRL	RL	TMD$_R$	LDC$_{0.5}$	PXV$_{CSA}$	NXV$_{CSA}$	DXV$_{CSA}$	RC	H	B	LA	SLA	LMR	SMR	RMR	RLR	Pn	E	Gs	Ci	Y$_L$
Root morphology	MRD	Mean root diameter (mm)	**-0.74**	-0.50	-0.19	**-0.94**	-0.45	-0.22	-0.22	-0.33	-0.04	0.18	0.11	-0.02	-0.18	-0.20	0.21	**-0.61**	-0.02	-0.13	-0.16	-0.07	-0.43
	SRL	Specific Root Length (m g^{-1})		0.58	-0.45	0.63	0.38	0.39	0.03	0.21	0.01	-0.23	-0.15	-0.18	0.30	0.24	-0.31	**0.85**	0.34	0.42	0.48	0.12	0.48
	RL	Root Length (cm)			-0.12	0.51	0.30	0.04	0.23	0.24	0.38	0.44	0.26	-0.50	0.15	0.13	-0.16	**0.62**	0.21	0.26	0.33	-0.09	0.25
	TMD$_R$	Tissue mass density of root (g cm^{-3})				0.28	-0.07	-0.29	0.16	0.03	0.08	0.22	0.12	0.16	-0.21	-0.03	0.16	-0.46	-0.50	-0.56	-0.55	-0.15	-0.12
	LDC$_{0.5}$	Length of diametric class > 0 < 0.5 mm (%)					0.44	0.20	0.22	0.31	0.08	-0.07	-0.06	0.02	0.12	0.20	-0.17	0.54	0.06	0.16	0.17	0.01	0.45
Root anatomy	PXV$_{CSA}$	Proportion of xylem CSA dedicated to vessels (%)						0.30	**0.64**	**0.92**	0.26	0.11	0.29	0.09	0.44	0.14	-0.36	0.28	0.02	-0.04	-0.05	-0.20	0.08
	NXV$_{CSA}$	Number of xylem vessel per mm^2 of xylem CSA							-0.52	-0.06	-0.34	-0.33	-0.33	-0.08	-0.10	-0.21	0.16	0.56	0.25	0.24	0.17	-0.03	0.26
	DXV$_{CSA}$	Mean diameter of xylem vessels (mm)								**0.86**	0.50	0.37	0.51	0.10	0.43	0.33	-0.44	-0.17	-0.10	-0.20	-0.15	-0.20	-0.10
	RC	Relative hydraulic conductivity (mm^{4}10^6)									0.42	0.24	0.44	0.12	0.53	0.23	-0.46	0.04	-0.10	-0.17	-0.15	-0.22	-0.08
Plant growth and architecture	H	Height (cm)										**0.81**	**0.91**	-0.21	**0.71**	**0.65**	**-0.77**	-0.27	0.03	-0.26	-0.21	-0.60	-0.16
	B	Biomass (g)											**0.89**	-0.39	0.36	0.40	-0.43	-0.32	0.06	-0.29	-0.24	**-0.66**	-0.22
	LA	Total Leaf Area (cm^2)												-0.11	**0.70**	0.53	**-0.71**	-0.38	0.04	-0.37	-0.33	**-0.70**	-0.19
	SLA	Specific Leaf Area (m^2 Kg^{-1})													0.10	-0.21	0.02	-0.22	-0.38	-0.23	-0.30	0.26	0.15
	LMR	Leaf Mass Ratio (kg kg^{-1})														0.57	**-0.93**	-0.11	0.16	-0.07	-0.04	-0.39	-0.01
	SMR	Stem Mass Ratio (kg kg^{-1})															**-0.83**	-0.15	0.17	-0.12	-0.07	-0.47	0.05
	RMR	Root Mass Ratio (kg kg^{-1})																0.14	-0.18	0.10	0.06	0.48	-0.02
	RLR	Root Lenght Ratio (root length/plant mass) (m g^{-1})																	0.36	0.54	0.54	0.23	0.46
Plant physiology	Pn	Net photosynthesis rate (mmol CO$_2$ m^{-2} s^{-1})																		**0.70**	**0.70**	-0.27	0.22
	E	Evapotranspiration rate (mmol H$_2$O m^{-2} s^{-1})																			**0.96**	0.39	0.17
	Gs	Stomatal conductance (mmol H$_2$O m^{-2} s^{-1})																				0.39	0.25
	Ci	Internal concentration of CO$_2$ (ppm)																					0.35
	Y$_L$	Leaf water potencial (Bar)																					

Chapter 7

Discusión general

Del carácter multifactorial a las respuestas múltiples

En la presente memoria se ha realizado especial hincapié en la dificultad de establecer una teoría general para describir la naturaleza de la compactación del suelo y sus efectos sobre el desarrollo de las plantas. Las variables que consideramos descriptoras de la compactación muestran un juego sutil de interacciones que obligan a hablar en términos cuantitativos y de dependencia con el caso de estudio. Durante el desarrollo de los capítulos, las variables más importantes que se han empleado como indicadoras de la compactación, densidad aparente y resistencia a la penetración, muestran una fuerte relación entre ellas y con el contenido de agua (Fig. 1). La densidad aparente es una medida indirecta de la porosidad, de forma que un incremento en la densidad aparente produce una disminución de la porosidad, como fue mostrado en el capítulo 4. Por tanto, seria de esperar que un incremento de la densidad aparente mostrara una relación inversa con el contenido de humedad, como efectivamente encontramos (Fig. 1). No obstante, en el capítulo 4 se mostró como a pesar de que en términos relativos (*Mass water content*, %) se observa una disminución con el aumento de la densidad aparente, en términos absolutos encontramos como el incremento de la masa de suelo por unidad de volumen también incrementa la masa de agua. Sin embargo, esta relación es de esperar que esta relación se vuelva negativa alcanzando valores elevados de densidad aparente, por la lógica disminución del volumen que puede ocupar el agua. Para terminar de relacionar estos conceptos debemos hablar del potencial matricial del suelo. El potencial mátrico mide la fuerza con la que son retenidas las moléculas de agua dentro de la matriz del suelo. Por tanto, al aumentar la densidad también aumentamos el potencial y con ello la dificultad de extraer el agua del suelo. Para finalizar esta síntesis de interrelaciones entre las variables fundamentales de la compactación, habría que comentar que la resistencia a la penetración, a pesar de ser un buen predictor del estrés mecánico potencial al que puede estar sujeto el crecimiento de una raíz, está muy influida por el contenido de humedad, haciendo difícil el uso de un valor de resistencia sin el contenido de humedad al que se midió(Apéndice capítulo 4).

Además, la granulometría y la pedregosidad de un suelo pueden estar incrementando los valores de resistencia, sin que sean relevantes desde el punto de vista del crecimiento radicular en la exploración efectiva de un suelo. Por ello, densidad, resistencia y humedad deben ser tomadas como variables inseparables para describir el estado de compactación de un determinado tipo de suelo.

Con todo esto se pretende subrayar el efecto potencial que la compactación tiene sobre las relaciones hídricas planta-suelo y la dificultad de separar, los efectos por estrés hídrico de los efectos por estrés mecánico ambos efectos.

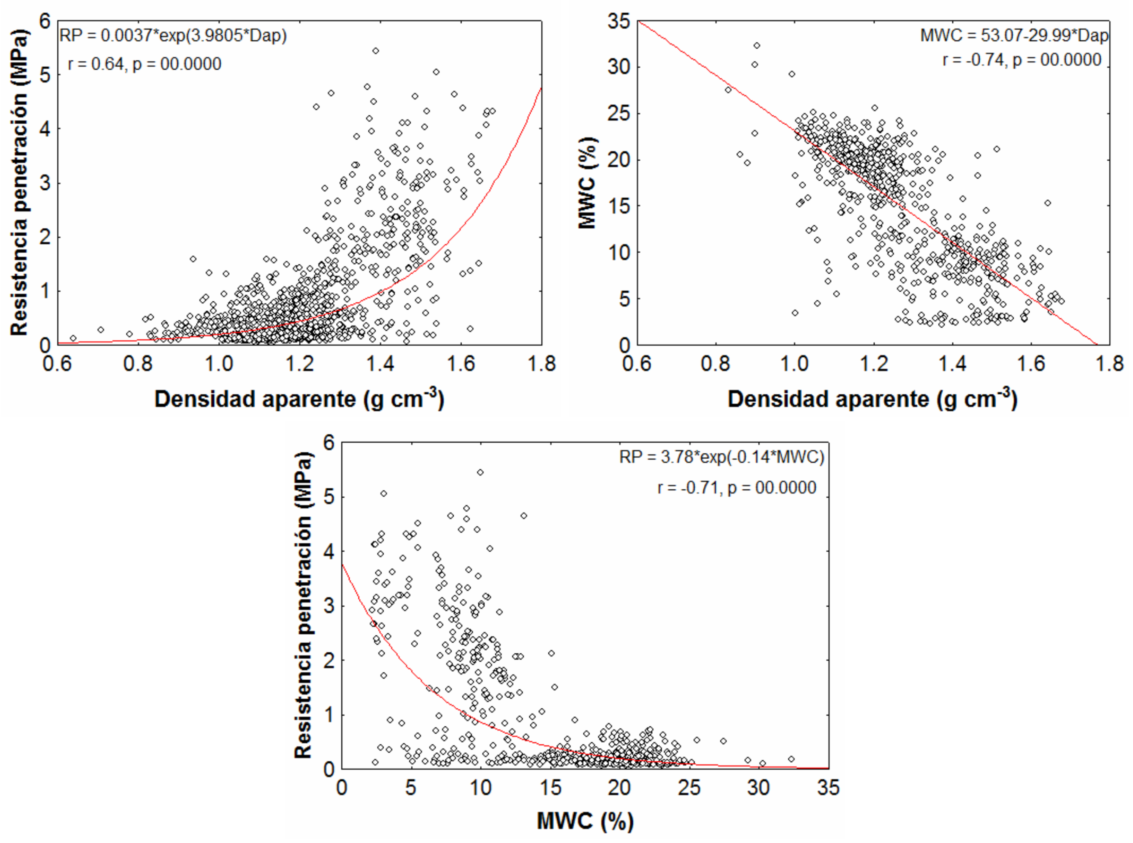


Figura 1. Relaciones densidad aparente, resistencia a la penetración y contenido gravimétrico de agua (MWC, *Mass Water Content*). En cada caso se muestra la ecuación de regresión con su coeficiente y nivel de significación asociado (N=1067).

El resultado de la interacción de estas variables (densidad aparente, resistencia a la penetración y contenido de humedad) vendrá determinado a su vez por el grado de intensidad de la perturbación y el tipo de suelo, atendiendo sobre todo a su granulometría y al contenido de materia orgánica. Así, el procedimiento empleado para crear los tratamientos de compactación en los experimentos de invernadero (Capítulos 3-6) pretendía simular diferentes grados de perturbación para conocer sus efectos sobre el rango. En los capítulos 3 y 4, la compactación manual nos permitió obtener un rango de compactación moderada para comenzar a explorar los efectos sobre el crecimiento de las plantas desde la inexistencia de compactación a valores medios (1.16 ± 0.12 g cm^{-3} de densidad aparente o 0.46 ± 0.40 MPa de resistencia a la penetración). El empleo de un sistema mecánico de compactación por percusión (Capítulos 5 y 6) nos permitió explorar los efectos de la compactación en un rango más amplio (1.47 ± 0.11 g cm^{-3} y 2.78 ± 1.18 MPa) así cómo observar la respuesta diferencial de dos texturas distintas sujetas a un mismo tipo de perturbación (Capítulo 6).

Por un lado, la respuesta a la perturbación varía en función a la granulometría: dado que la densidad de las partículas de arena es mayor, tenderá a producir valores de densidad aparente mayores, además de que su capacidad de reorganización es mayor dada su alta macroporosidad. Este fenómeno fue explicado por Whalley et al. (2000) de forma que los suelos arenosos redistribuyen la compactación de forma densa y homogénea a lo largo del perfil; mientras que los suelos francos presentan una mayor capacidad al distribuir las perturbaciones en 2 capas distintas: una superficial de macroporos y una profunda de microporos.

Por último, en el capítulo 1 comprobamos como el rango de compactación empleado en condiciones controladas es representativo del que se encuentra en condiciones naturales (1.43 ± 0.08 g cm^{-3} y 2.30 ± 0.90 MPa). Además, se introduce un componente muy importante en el proceso de compactación: la materia orgánica. En dicho trabajo, resulta sorprendente el importante papel que desempeña la cobertura arbórea como de-compactadores mediante la adición de materia orgánica, fenómeno que se discutirá en un apartado más adelante.

Por todo esto, el carácter multifactorial de la compactación debe ser tenido en cuenta para entender en qué condiciones concretas de estrés se encuentran las plantas y cuáles pueden ser las causas de las múltiples respuestas dadas por ellas.

Modelo general de respuesta del crecimiento a la compactación

En esta tesis se ha testado el comportamiento de un total de 19 especies de plantas vasculares, centrándonos fundamentalmente en la respuesta de leñosas (18) pertenecientes a ecosistemas mediterráneos o al menos presentes en ellos: *Quercus ilex*, *Q. coccifera*, *Q. suber*, *Q. faginea*, *Q. canariensis*, *Q. pyrenaica*, *Pistacia lentiscus*, *P. terebinthus*, *Rhamnus alaternus*, *Frangula alnus*, *Olea europaea*, *Fraxinus angustifolia*, *Ceratonia siliqua*, *Cercis siliquastrum*, *Pinus sylvestris*, *Prunus lusitanica*, *Ailanthus altísima y Nerium oleander*. El estudio de las herbáceas se ha restringido a dos especies *Medicago sativa* y *Nicotiana tabacum*.

La variabilidad de respuestas en función al rango ha sido muy elevada, como se pone de manifiesto en los capítulos 3 y 4. Las respuestas diferenciales frente a la textura han sido mostradas en el capítulo 6. Además es de gran relevancia el fuerte compromiso que se establece entre la respuesta a la compactación y la interacción con otros factores ambientales (Capítulos 4 y 5).

En cualquier caso, las respuestas encontradas por las especies pueden resumirse en dos ideas generales. La primera pone énfasis en la alta dependencia del rango, ya que la compactación además de la percepción general de producir efectos negativos en el crecimiento, también produce efectos positivos en valores moderados. Como se mostró en los capítulos 3 y 5, bajo condiciones óptimas de luz y agua, valores intermedios de densidad aparente pueden beneficiar el crecimiento de las plantas. Este fenómeno es explicado en la literatura por un mayor contacto raíz-suelo, que influye de manera decisiva en el transporte de nutrientes y en la toma de agua. Así, en condiciones óptimas de luz y agua podemos postular un modelo general de respuesta a la compactación que adopta la forma de una campana, donde el óptimo de crecimiento se alcanza en los valores intermedios de compactación (Fig. 2).

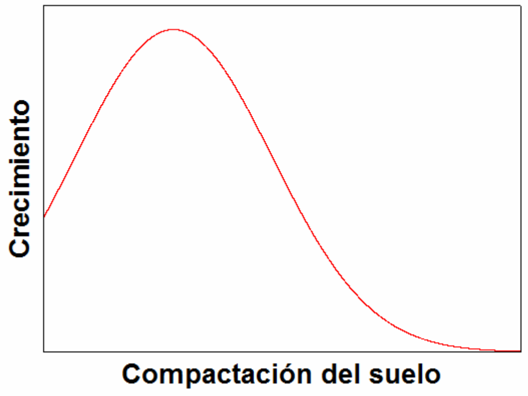


Figura 2. Modelo general de respuesta del crecimiento vegetal a la compactación del suelo. La respuesta del crecimiento se aproxima a un modelo binomial donde se alcanza un óptimo en valores intermedios de compactación.

Los efectos negativos de la compactación deberán ser explicados como una limitación al crecimiento radicular y de ahí, una disminución en la exploración del suelo y la explotación de los recursos disponibles. Así, la segunda idea general sobre la respuesta a la compactación remarca el importante efecto sobre el funcionamiento de la raíz, que abarca cambios morfológicos (disminución de la tasa de elongación, disminución de la longitud específica radicular o disminución de la proporción de raíces finas), fisiológicos (disminución de la respiración radicular, transporte de agua y nutrientes) y anatómicos (cambios en la tasa y plano de división celular, grosor del córtex, número y diámetro de los vasos xilemáticos, etc.). La señalización hormonal raíz-porción aérea es la encargada de comunicar los impedimentos a los que se enfrenta la raíz a lo largo de su desarrollo (Young et al., 1997), ya que las limitaciones en el abastecimiento de agua y nutrientes son determinantes para el crecimiento aéreo. Algunas hipótesis de cuáles pueden ser los mecanismos se explicarán en un apartado posterior.

La respuesta a la compactación ha sido muy variable, dependiendo de las especies. Algunas especies no manifiestan efectos beneficiosos de la compactación moderada sobre el crecimiento, mientras que otras a bajos rangos responden con un incremento del crecimiento (Capítulo 3). En general para la mayoría de las especies se produce una distorsión de la arquitectura de planta completa. El comportamiento de cada especie a la compactación es explicado en la literatura como un rasgo genético (Materechera et al., 1991). Por otro lado, resulta lógico encontrar la habilidad para explorar el suelo como un rasgo adquirido en el transcurso de la evolución, siendo un factor de selección para la colonización de nuevos hábitat y expansión de la especie.

La compactación como modificador de la arquitectura de las plantas

Un resultado importante que se extrae de forma consistente a lo largo de los diferentes capítulos es que la compactación modifica la arquitectura de las plantas. Además, como se vio en el capítulo 4, existe una relación muy estrecha entre estos cambios morfológicos y la supervivencia a la sequía; por lo que la compactación puede favorecer la resistencia o aumentar la susceptibilidad de las plantas a determinados factores ambientales. Por otro lado, la tasa de crecimiento relativo se relaciona positivamente con el área foliar y la proporción de hojas y tallo, pero negativamente con la proporción de raíz. En cambio, la relación de estas variables con el tiempo de supervivencia es la contraria. Por lo que podemos vincular la tasa de crecimiento de forma negativa con la capacidad de las plantas para sobrevivir a factores ambientales muy dependientes de la morfología de las plantas, como es el caso de la sequía (Fig. 3). La compactación tiene un papel importante en estas relaciones. Por un lado, la compactación afecta a la distribución de biomasa, en concreto aumentando la proporción de raíz y esto tiene consecuencias aumentando la tasa de supervivencia. Por otro lado, el aumento de compactación, puede incrementar el contenido de agua total y de esta forma favorecer la supervivencia. En este sentido, sería lógico esperar un modelo de campana para la relación compactación-supervivencia, donde a valore elevados de compactación ya no existiesen efectos beneficiosos en el almacenamiento de agua y comenzasen a actuar otros factores como estrés mecánico (resistencia) e hídrico (potencial mátrico).

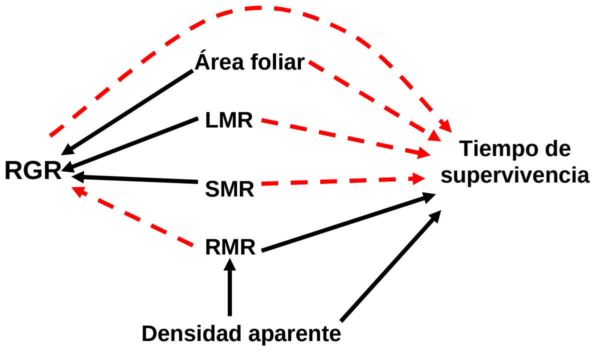


Figura 3. Compromiso entre crecimiento y supervivencia mediado por la arquitectura de la planta, y el papel que la compactación del suelo juega en este compromiso.

Interacción con otros factores

A pesar de que las aproximaciones experimentales de invernadero nos permiten aislar la compactación para entender su funcionamiento, en la naturaleza la encontraremos en interacción con otros factores. En el capítulo 5 se ha mostrado cómo la compactación tiene un efecto diferente en función a las condiciones de luz, agua y en la interacción con el estrés mecánico. Así, bajo condiciones óptimas de luz, agua y en ausencia de estrés mecánico, la respuesta de las plantas seguiría el modelo binomial anteriormente mencionado. Sin embargo, las limitaciones de luz y agua producen un fuerte compromiso con la compactación, modificando este modelo general (Fig. 3).

Figura 4. Modelo general modificado de los efectos de la compactación del suelo. En a (izquierda) puede verse la respuesta en campana referida a condiciones óptimas de luz y agua. En b (centro) se muestra la modificación del modelo binomial en uno lineal negativo bajo condiciones de limitación de agua. En c (derecha) se muestra una respuesta nula bajo condiciones de limitación de luz.

Si entendemos la compactación del suelo como una limitación en la disponibilidad y toma de agua, un bajo régimen hídrico va a limitar aún más el agua disponible para el crecimiento. Teniendo en cuenta el aumento del potencial matricial del suelo producido por compactación, puede producirse un colapso en el transporte de agua y nutrientes de la matriz del suelo al interior de la raíz. El resultado es un efecto negativo sobre el crecimiento, aumentando en intensidad mientras más alto es el nivel de compactación (Figura 3b). En condiciones limitantes de luz, como es el caso del crecimiento bajo sombra, las plantas emplean más recursos en aumentar la proporción de hojas en detrimento del crecimiento de la raíz. Dado que las demandas evaporativas disminuyen bajo sombra, los efectos limitantes de la compactación a la toma de agua sólo se producirían cuando la proporción radicular comenzase a ser insuficiente para satisfacer las necesidades hídricas. Por todo esto, la condiciones limitantes de luz podrían llegar a eliminar el efecto negativo de la compactación del suelo sobre el crecimiento (Fig. 3c).

Los resultados del capítulo 6 provienen de un experimento de mayores dimensiones en el que se pretendía evaluar la respuesta de diferentes grupos funcionales (árboles, matorrales y herbáceas) a la compactación de 2 tipos de suelo contrastantes (franco y franco-arenoso). Como se puede observar en el suelo franco la compactación no afecta a la tasa de crecimiento relativo (RGR) para ninguna de las tres especies (Fig. 4). Sin embargo, en el franco-arenoso, dos especies (*F. angustifolia y N. oleander*) presentan una disminución de RGR con la compactación. Así, los resultados obtenidos ponen de manifiesto la alta dependencia de la respuesta a la compactación en función a la textura.

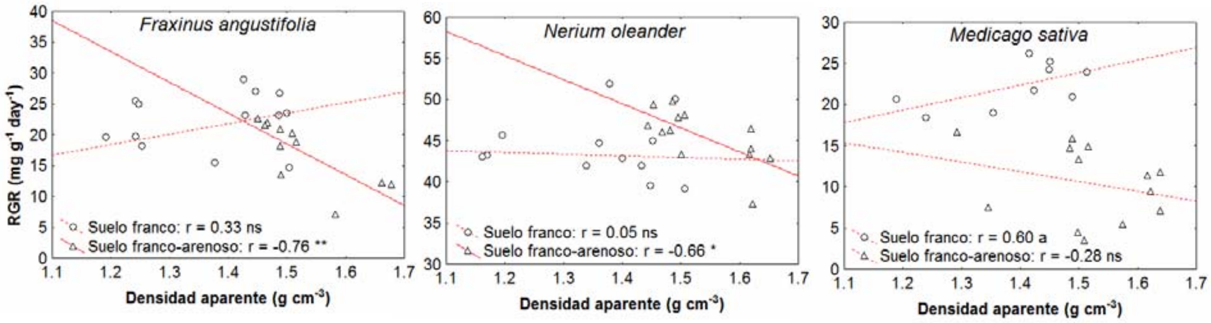


Figura 5. Efecto de la compactación sobre la tasa de crecimiento relativo (RGR, Relative Growth Rate) en función a al textura. Resultados de un experimento de compactación en 2 suelos naturales de textura diferente (franca y franco-arenosa) empleando 3 grupos funcionales distintos. Un árbol (*Fraxinus angustifolia*), un matorral (*Nerium oleander*) y una herbácea (*Medicago sativa*). En cada caso se muestran el coeficiente de regresión y su nivel de significación, indicado por: ns, no significativo; a, marginalmente significativo; *, significativo *P*<0.05; **, significativo *P*<0.001.

Mecanismos fisiológicos de respuesta a la compactación

En el capítulo 6 se ha realizado una aproximación a los mecanismos que subyacen al efecto de la compactación sobre el desarrollo vegetal. En dicho trabajo se evaluaron variables fisiológicas (fotosíntesis, transpiración, potencial hídrico), crecimiento y distribución de biomasa, morfología y anatomía de raíz.

Los resultados presentados ponen de manifiesto una secuencia de mecanismos en la respuesta a la compactación. En primer lugar, hay que remarcar el hecho de que la compactación genera dos tipos de estrés: mecánico sobre la raíz e hídrico sobre la planta completa. La deficiencia en la toma de nutrientes no debe ser considerada como una entidad aparte, ya que viene condicionado por el transporte de agua. Así, podríamos establecer una secuencia simplificada de respuestas en escala temporal (Fig. 5):

1. El proceso de compactación tiene fundamentalmente tres efectos sobre el suelo: aumento de la dureza, disminución de la aireación y aumento del potencial mátrico.
2. Estas modificaciones del suelo en el que se desarrolla la raíz van a provocar cambios en la morfología debido a la impedancia mecánica. Se produce una disminución de la elongación, la producción de raíces finas y por tanto disminuye la exploración efectiva del suelo. En este proceso pueden concurrir efectos de la baja aireación sobre la tasa de respiración, con el consiguiente efecto sinérgico sobre la reducción de la tasa de elongación radicular.
3. Las limitaciones a la exploración del suelo (tanto por disminución de raíces finas como por una menor exploración en profundidad) llevarían a una disminución de la toma de agua y esto ocasionaría un déficit hídrico.
4. Dado que existen unas demandas evaporativas para el crecimiento aéreo unido a un déficit en el abastecimiento de agua por parte de la raíz, la llegada a una situación de estrés hídrico provoca una señal de respuesta mediada por reguladores del crecimiento, fundamentalmente ABA y etileno (Moss et al., 1988; Hussain et al., 2000).
5. La función del etileno será la de inhibir el crecimiento aéreo, mientras que la del ABA es la de disminuir la conductancia estomática fundamentalmente.
6. El resultado de ambas señales es un ciclo de retroalimentación dirigido a disminuir las pérdidas de agua tanto a nivel morfológico (disminución del área foliar) como a nivel fisiológico (disminución de la transpiración).

El efecto indirecto del cierre estomático puede ser a su vez causa y efecto de la disminución del crecimiento bajo condiciones de compactación.

7. La plasticidad concreta de la especie frente a una situación de estrés hídrico llevará a tolerar o evitar la sequía (Capítulo 4). A nivel radicular existe una alta posibilidad de responder mediante una aclimatación anatómica, encaminada a incrementar el transporte de agua para no colapsar el crecimiento. La disminución del córtex de las raíces con función absorbente disminuye la resistencia hidráulica del agua desde el suelo al interior de la raíz (Steude, 2000). Además la fuerza necesaria para obtener agua de un suelo compactado es incrementada debido al potencial mátrico. Una respuesta potencial sería la disminución del diámetro de los vasos xilemáticos para cumplir una doble función: aumento de la capilaridad y transporte hídrico bajo mayores márgenes de seguridad (evitación de la cavitación).

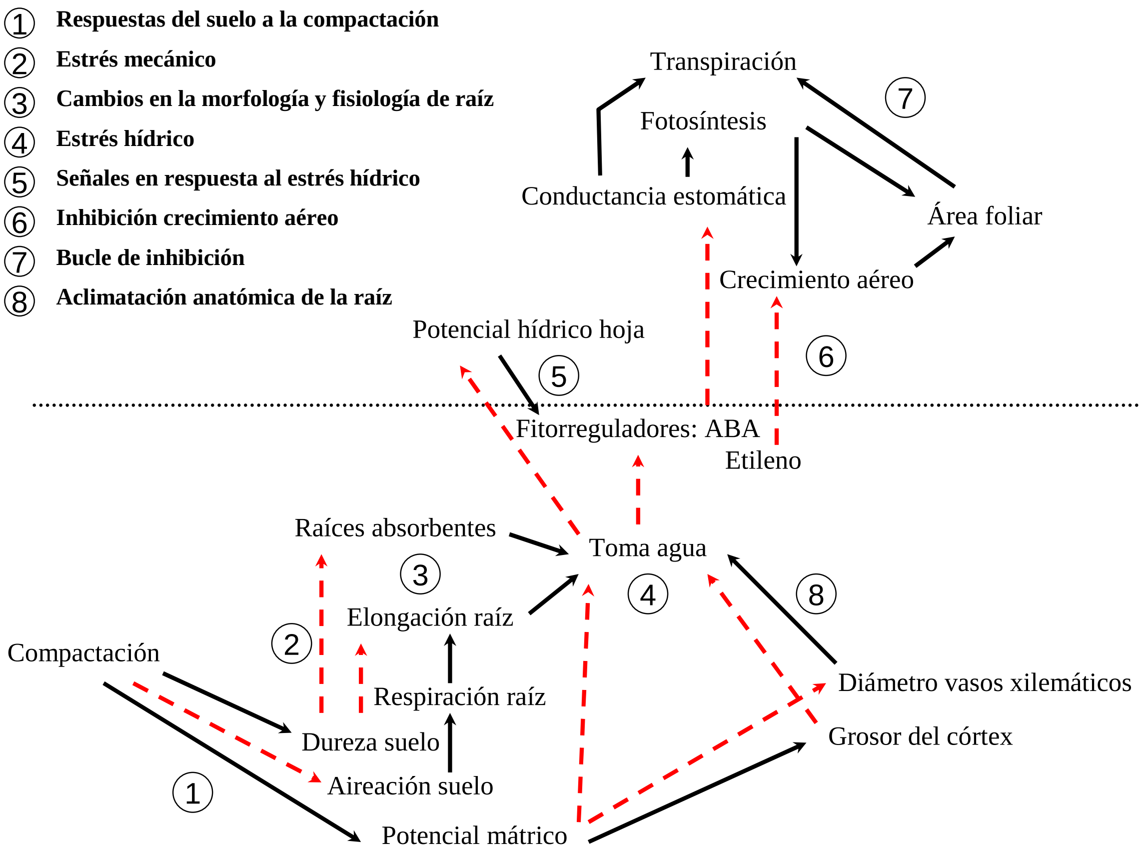


Figura 6. Mecanismos de acción de la compactación del suelo sobre el desarrollo de las plantas. Flechas discontinuas indican efecto negativo, líneas continuas efecto positivo.

El efecto de la cobertura arbórea sobre la compactación

Es habitual referirse a la compactación como un proceso de perturbaciones de muy diverso origen: maquinaría, ganado, incendios, sequía, etc. En la naturaleza la formación de la estructura del suelo tiene una alta dependencia con la actividad biológica. De otro modo, los procesos formadores del suelo darían lugar a una matriz resultado de agentes erosivos (escorrentía, viento, hielo), que con bastante probabilidad producirían un suelo con estructura compactada. En el capítulo 2, la descripción de los patrones espaciales de la compactación nos da la pista de que en el proceso de formación de la estructura la cobertura arbórea juega un papel fundamental. La especie *Quercus ilex* muestra un papel muy importante como ingeniero del ecosistema (Gallardo, 2003). Las propiedades físicas del suelo bajo su copa muestran diferencias significativas con respecto al suelo lejos de su alcance. La adición de hojarasca y su consiguiente descomposición, aumenta considerablemente el contenido de materia orgánica. El efecto sobre la estructura del suelo es visible como una disminución de la densidad aparente. A su vez la resistencia a la penetración es también menor, dada la adición de materia orgánica y el aumento de la retención de agua. En este sentido, parece que el efecto de la encina es de-compactar el suelo. Esto tiene implicaciones sobre la productividad de herbáceas, sobre todo al disminuir la limitación del crecimiento en profundidad. Sin embargo, el efecto de *Pinus pinaster* sobre la estructura del suelo y la compactación es muy bajo. Al mantener una compactación superficial elevada, explicada por la escasa tasa de mineralización de su hojarasca, la implantación de herbáceas también es menor. Si añadimos el comportamiento alelopático de la hojarasca, el efecto es producir un suelo desprovisto de vegetación. Así, en sentido opuesto a como lo hace la encina, el pino piñonero no modifica la compactación del suelo. Por tanto, el fenómeno de la compactación en condiciones naturales queda fuertemente vinculado al papel que la vegetación ejerce sobre él, disminuyendo, manteniendo o incrementando la compactación.

Conclusiones

Las conclusiones más importantes de esta tesis sobre los efectos de la compactación del suelo y las implicaciones ecofisiológicas sobre el desarrollo vegetal subrayan:

1. La compactación del suelo muestra un patrón espacial en la naturaleza íntimamente relacionado con la cobertura arbórea y dependiente de la especie.
2. *Quercus ilex* tiene un importante papel como ingeniero del ecosistema. Produce efectos visibles sobre las propiedades físicas del suelo disminuyendo la densidad aparente. Sin embargo, *Pinus pinaster* no modifica las propiedades físicas del suelo. Esta interacción de la cobertura arbórea con las propiedades físicas del suelo tiene implicaciones en la producción de herbáceas.
3. La compactación moderada del suelo tiene efectos positivos sobre el crecimiento, produce cambios en la arquitectura de la planta, lo que puede tener implicaciones ecológicas aumentando el tiempo de supervivencia a la sequía.
4. El modelo general de respuesta a la compactación sobre el crecimiento vegetal adopta la forma de campana donde a valores intermedios de compactación se alcanza un óptimo. Dicho modelo es modificado en interacción son otros factores ambientales: luz, agua y estrés mecánico.
5. Los principales efectos de la compactación del suelo sobre el desarrollo vegetal se resumen en 2 respuestas: cambios en la morfología y anatomía radicular y una distorsión de la arquitectura de la planta, todo lo cual tiene implicaciones sobre el funcionamiento de planta completa.
6. Los mecanismos de acción de la compactación sobre el desarrollo vegetal implican una serie de respuestas secuenciales: modificación de la morfología radicular en respuesta al estrés mecánico, inhibición del

crecimiento aéreo en respuesta al déficit hídrico y cambios plásticos para tolerar y/o evitar los efectos del estrés por compactación.

7. La compactación del suelo debe ser tenida en cuenta en los modelos de establecimiento y regeneración de las especies dado que podría actuar como filtro ambiental en la germinación y emergencia, e influir en la susceptibilidad a otras fuentes de estrés como es el caso de la resistencia a la sequía.

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Acknowledgments

Agradecimientos

Sería muy difícil expresar en estos párrafos mi agradecimiento a toda la gente que me ha ayudado a lo largo del camino de la tesis. En este periodo de 4 años y medio, que no es poco, se aprende a superar obstáculos, tanto en el ámbito de la investigación, como los de la vida fuera de ella. Probablemente los primeros queden aquí recogidos, sin embargo los segundos son de difícil descripción. Ahora mismo me encuentro exhausto, porque cometí el error de dejar este apartado para el final, y me enfrento al cierre de este documento que debe responder al trabajo de formación del periodo doctoral. Teóricamente, debo ser capaz de demostrar todo lo que he madurado, lo que puedo llegar a dominar el tema y ante todo, qué he aprendido. Probablemente ésa sea la clave para poder escribir este apartado, dedicar estas líneas a lo que he aprendido de las personas que han colaborado, a veces sin saberlo, a que en este momento pueda decir, estoy acabando.

El primero que aparece en mi mente, y no es casual, es Rafael Villar. Creo que los que se han enfrentado a la redacción de una tesis me entenderán sin problema. Tu director es la persona que en el tiempo en el que dura la tesis más veces ocupa tu cabeza, tu agenda, tu buzón de correo, las utópicas vacaciones, y en general, todos aquellos momentos en los que te planteas si existe algo fuera de esta vida. Honestamente, puedo decir que de lo que conozco, creo que me llevé lo mejor. No sé si es fruto de esa máxima popular de "más vale malo conocido.." pero sin duda puedo reconocer lo que he aprendido de él. Rafa, gracias por confiar en mí al nivel que los has hecho, por ser paciente con mi carácter, con mis otras vocaciones y por haber ayudado tanto a que la ciencia me parezca tan fascinante. He aprendido a disfrutar de los buenos resultados, a saber aceptar los malos y a no parar de generar datos. Tu pasión por lo que haces nunca me ha dejado indiferente, de hecho he de confesar que esto es lo que más me ha calado. Puedes estar tranquilo, la investigación es vocacional, pero tú has conseguido alimentarla con buen hacer. Gracias por haberme brindado esta oportunidad, esto me acompañará siempre.

Mis compañeros de departamento son los que en segundo lugar vienen a mi mente. Por orden cronológico creo que Carmen Ordoñez fue la primera. Gracias Carmela, me has visto en todos los estados posibles, y ha sido una alegría tenerte ahí en todo momento, de verdad gracias. Joaquín Reyes ha sido la persona que más carcajadas me ha arrancado de este grupo, compensado por las inmensas horas de consejos estadísticos que yo le he arrancado a él. Paco Conde ha sido una conexión con mi otra gran pasión; en las largas horas de trabajo de invernadero no hemos parado de hablar de lo que habría sido vivir de la música. A Vico le debo la paciencia de haber compartido tutor y cubículo conmigo, ha sido genial. A Elena Suárez los buenos momentos de "riegos intensivos" durante un verano abrasador. A Pepón Vacas las horas de cosechas, las risas midiendo con el penetrómetro y la redefinición de algunos conceptos. A David Ariza por nuestros momentos críticos y de crítica. A todos los que han pasado por el grupo: María Silvestre, Raquel Casado, Cristina Gómez, David Gómez, Laura Gil, Manolo Olmo, Jaime Muriel y Sergio De Haro; y a los que continúan que no desistan Pilar Fernández, Salvador Arenas, Juan Carlos Bonillo, Bárbara López y Andrés Cortés. No me puedo olvidar de los alumnos colaboradores en los que se sustenta gran parte de la investigación de este país: Nani, Cristina, Emilio y Fernando.

A mi familia le debo la comprensión y el ánimo constante. Gracias Mamá por apoyarme en cada decisión que he tomado. Gracias Papá por valorar el esfuerzo que me ha costado todo lo que hecho. A Titere, Miguel, Juan Antonio, Bibel y Carlos por estar desde la distancia mostrando unidad familiar. A Álvaro por haber alimentado la vía musical, por ayudarme en algunas etapas de los experimentos y por aguantarme estando tan cerca.

A la familia Baet Klezmer Band: Jose, Fale, Anke, Kiqe, Nuria, Pove, Carlos y Manolo; gracias por haber sido el contrapunto a este mundo tan serio, dándole el ritmo adecuado y sacando de mí al violinista!! La tesis no habría salido adelante sin la música, sin la adrenalina de conciertos y sin tantas horas despertando emociones.

A mis amigos de toda la vida tengo que agradecer el no pensar que estaba loco dedicando tanto tiempo a esto, y de pensarlo, ayudarme para que no fuese algo irreversible. Gracias Ocaña, Kris, Ana, Palma y Ana, que sin duda los años harán todas estas cosas más pequeñas.

De mi estancia en Utrecht me llevo.. me lo llevo todo! La pasión por el trabajo bien hecho, el buen ambiente de trabajo, el diálogo científico, la gente trabajadora y esos invernaderos. Holanda ha sido la inspiración y el contexto donde ha sido posible escribir la tesis. Gracias a Niels Anten por la confianza y libertad en el trabajo desarrollado juntos. A Feike Schieving por sus inspiradoras conversaciones. A toda la gente de Plan Ecology & Biodiversity porque, sin saberlo, me han dado el contexto para ser productivo. A Elisabeth Giddings por su ayuda en los experimentos de la estancia y por haberse dejado impregnar por lo bonito de la investigación. A Anne Sandbrink y Bart Vollaard por haber sido, entre mucho, el contrapunto musical en versión holandesa. A Danaë Rozendaal por la sorpresa de conocer a un alter ego, ecóloga y violinista. A Isa, Carlos y Tere por constituir la colonia. A Fani por los breaks que me han dado la calma en los momentos de escritura. A Chiara por saber sacarme de todo esto. Muy especialmente tengo que agradecer a Claudia Soliz-Gamboa por haber compartido la misma etapa vital en el despacho, el estrés, los miedos, las dudas, nuestros desayunos y una buena onda que nos ha empujado a acabar.

En fin, espero no olvidarme a nadie, y de hacerlo, lo siento. Ante todo, gracias, gracias por haber estado apoyando al que escribe a finalizar algo que parecía imposible.

David Alameda Márquez
Córdoba, 25 de abril de 2010

Curriculum Vitae

David Alameda Márquez was born on 6th of May 1982 in Córdoba, Spain. In 2000 he obtained his high school qualification at the I.E.S. "Gran Capitan" in Córdoba. In the same year he started his Biology studies at Cordoba University. In 2004 he studied a semester at Oviedo University under the SICUE-Seneca agreement, where he made his first contact with ecophysiology discipline. After he obtained his Bachelor's Degree in 2005, he started a research project on ecophysiology with the Ecology group of Cordoba University. In 2007 he obtained his degree in Violin at Professional Conservatorium of Cordoba; afterwards he started his degree in History and Science of Music at La Rioja University (Spain). In the same year he cursed the PhD program "Science and Forestry Engineering" carrying out his thesis' experiments and continuing the research line started during his Bachelor's thesis. In 2009 he spent one year at Utrecht University to conduct the final experiment and to finish writing up his thesis. The result of these four years' work appears in the current book.

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