TESIS DOCTORAL

ELENA LOZANO GUARDIOLA

Sensibilidad de la glomalina a los efectos provocados por el fuego en el suelo y su relación con la repelencia al agua en suelos forestales mediterráneos

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Sensitivity of glomalin to fire effects on soil and its relationship with water repellency in Mediterranean forests soils

Departamento de Agroquímica y Medio Ambiente. Universidad Miguel Hernández
Directores: Jorge Mataix Solera, Victoria Arcenegui Baldó y Jorge Mataix Beneyto
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La tesis ha sido dirigida y revisada por los profesores abajo firmantes, el Dr. Jorge Mataix Solera, la Dra. Victoria Arcenegui Baldó y el Dr. Jorge Mataix Beneyto de la Universidad Miguel Hernández de Elche (Alicante).

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Elche, septiembre 2015

El doctor Ignacio Gómez Lucas, Director del Departamento de Agroquímica y Medio Ambiente de la Universidad Miguel Hernández de Elche,

CERTIFICA

Que la memoria adjunta, con título *"Sensibilidad de la glomalina a los efectos provocados por el fuego en el suelo y su relación con la repelencia al agua en suelos forestales mediterráneos"*, presentada por Elena Lozano Guardiola, ha sido realizada en el Departamento de Agroquímica y Medio Ambiente de dicha Universidad.

Fdo.: Ignacio Gómez Lucas

Director del Departamento

Elche, septiembre de 2015

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Mamá, ya puedes preguntarme:

he acabado la tesis!!

A mi gran amiga
Mary Carmen Lax,

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RESUMEN / ABSTRACT

SENSIBILIDAD DE LA GLOMALINA A LOS EFECTOS PROVOCADOS POR EL FUEGO EN EL SUELO Y SU RELACIÓN CON LA REPELENCIA AL AGUA EN SUELOS FORESTALES MEDITERRÁNEOS

RESUMEN

Los suelos son imprescindibles para la vida, por lo tanto entender como funcionan y como responden a las perturbaciones es indispensable para llevar a cabo una correcta conservación y uso adecuado de los mismos. Las particularidades del clima mediterráneo, caracterizado por un periodo estival muy caluroso y seco, hacen que los incendios forestales y el aprovechamiento del agua sean dos de los principales factores ambientales que mayor interés están recibiendo en las últimas décadas en el sureste español.

En esta tesis, ambas temáticas han sido abordadas a través del estudio de un componente del suelo, la glomalina, una glicoproteína producida por los hongos micorrícicos arbusculares, que llega al suelo tras la muerte y descomposición de las hifas del hongo. La presencia de esta glicoproteína en el suelo está siendo muy estudiada debido a su contribución en la reserva de carbono y nitrógeno, a sus implicaciones en la mejora de la estructura (a través de la formación de agregados), a su sensibilidad a las perturbaciones debido a los diferentes usos y manejos, así como en la captación de elementos tóxicos del suelo entre otros. En esta tesis, la glomalina ha sido estudiada como componente del suelo que nos puede proporcionar información acerca del estado del mismo tras un incendio forestal y como posible factor implicado en la falta de afinidad del suelo por el agua, propiedad conocida como repelencia al agua en el suelo, “Soil Water Repellency” (SWR) en inglés, que puede manifestarse tanto en condiciones naturales como después de perturbaciones como las provocadas por el paso del fuego.

El objetivo general de esta *Tesis Doctoral* “*Sensibilidad de la Glomalina a los efectos provocados por el fuego en el suelo y su relación con la repelencia al agua en suelos forestales mediterráneos*”, es estudiar los efectos provocados por los incendios forestales en los stocks de glomalina, así como los factores implicados en la aparición de la repelencia al agua y las consecuencias en la comunidad microbiológica de suelos forestales Mediterráneos. La tesis se presenta estructurada en cuatro capítulos en formato artículos. Todos los capítulos han sido publicados como artículos en revistas internacionales de impacto como lo son “Land

Degradation and Development” (Capitulo 1), “Science of the Total Environment” (Capítulo 2), “Geoderma” (Capítulo 3) y “Journal of Hydrology and Hydromechanics” (Capítulo 4).

El fuego es un factor ecológico natural, que como fenómeno existe mucho antes de la existencia del hombre, de hecho, es considerado un factor primordial para el mantenimiento de la estructura y buen funcionamiento de los ecosistemas. Desgraciadamente, en las últimas décadas, determinados factores antrópicos como el abandono agrícola y las políticas de supresión del mismo entre otros, han hecho que se produzca un cambio tanto en el régimen como en la intensidad de los mismos. El suelo es un componente básico del ecosistema forestal del cual dependen el resto de componentes. El efecto inmediato del calor y las condiciones post-incendio provocan cambios físicos, químicos y biológicos en el. Cambios que dependiendo de determinados factores y condiciones ambientales, pueden perdurar durante años. Por ello, la evaluación de los suelos tras un incendio es indispensable para su correcta conservación y recuperación. Cobra así gran importancia la búsqueda de componentes o indicadores del suelo capaces de informarnos de su evolución y recuperación. En este sentido, en el Capítulo 1 se estudió la respuesta de la glomalina a la temperatura. Para ello se llevaron a cabo quemas experimentales de laboratorio a seis temperaturas (180, 200, 250, 300, 400 y 500°C) en diferentes suelos de la provincia de Alicante. Para poder evaluar la utilidad de la glomalina como indicador de severidad del fuego y la información adicional que puede proporcionar con respecto a otros parámetros, también se analizó la respuesta de la materia orgánica y la repelencia al agua en el suelo. En total se analizaron 8 suelos representativos de la región, seleccionados principalmente en base a sus diferencias en textura; 3 arenosos, 4 limosos y 1 arcilloso. La respuesta del contenido de glomalina a la temperatura varió de unos suelos a otros, observándose comportamientos similares entre los suelos con texturas parecidas. Otras propiedades del suelo como la estabilidad de agregados y el contenido de materia orgánica, también influyeron significativamente en la respuesta a las diferentes temperaturas. Sin embargo, la respuesta de la materia orgánica a la temperatura fue muy similar entre los diferentes suelos. La repelencia al agua no apareció en ninguno de los suelos tras los diferentes tratamientos, salvo en aquellos que ya la presentaban en condiciones naturales, desapareciendo totalmente superados los 200°C. Los resultados indican que el uso combinado de diferentes parámetros del suelo, sensibles a las temperaturas, podría proporcionar información muy útil acerca de la severidad del incendio sobre el suelo.

Los estudios de laboratorio bajo condiciones controladas son necesarios para poder entender mejor que es lo que sucede en campo, aunque evidentemente, los resultados nunca podrán ser 100% contrastables. Por ello, el siguiente paso fue analizar la respuesta de la glomalina tras el paso de un incendio forestal. En el Capítulo 2 se estudió la sensibilidad de la glomalina a los cambios producidos tras el paso del fuego bajo la influencia de dos tipos de vegetación (pinar y matorral). Los efectos inmediatos del fuego fueron estudiados en dos áreas diferentes: Gorga y Gata de Gorgos (ambas de la provincia de Alicante), mientras que los efectos a medio plazo se llevaron a cabo tan sólo en Gorga, para ello se instalaron parcelas que fueron monitorizadas trimestralmente durante un año. El contenido de glomalina en el suelo inmediatamente después del incendio tan sólo varió significativamente en las parcelas de matorral en la zona de Gorga, lo que es indicativo de una mayor severidad del incendio respecto al resto de parcelas. Los resultados del contenido de materia orgánica también mostraron una mayor severidad bajo matorral, esta vez en ambas zonas. La diferencia de los resultados sobre la severidad entre la materia orgánica y la glomalina pueden deberse a la respuesta a la temperatura de cada uno de los parámetros. Los efectos del fuego en la glomalina a medio plazo si que fueron evidentes en ambos tipos de parcelas. Los contenidos de glomalina en las parcelas control presentaron un comportamiento estacional muy variable, mientras que en las parcelas quemadas no variaron a lo largo del periodo estudiado. La falta de variabilidad puede atribuirse a una baja tasa de mineralización y producción nueva de glomalina.

Por otro lado, la glomalina ha sido relacionada con la aparición de la repelencia al agua en el suelo, propiedad cuya aparición en un ecosistema semiárido como el del sureste peninsular, cobra especial relevancia. El suelo es el encargado del almacenamiento y distribución del agua, por lo que la presencia de repelencia al agua en su superficie condicionará el desarrollo de la cubierta vegetal y la actividad microbiológica, y por tanto todos los factores que dependen de ésta, como los ciclos biogeoquímicos, decisivos para el correcto funcionamiento del ecosistema en general. En este contexto, no es de extrañar la especial atención e importancia que en las últimas décadas ha recibido el estudio de la pérdida de afinidad por el agua (repelencia al agua), que bajo determinadas condiciones ambientales, manifiestan algunos suelos. La repelencia al agua es un fenómeno muy complejo cuya aparición puede estar ligada a un gran número de factores como: especie vegetal, materia orgánica, textura, pH, humedad, así como los incendios forestales etc. La repelencia es una propiedad muy compleja y difícil de estudiar con importantes repercusiones (negativas y positivas) a nivel ambiental. Estudiar las causas y las implicaciones ecológicas de su aparición en condiciones naturales, es crucial para poder entender mejor sus posibles efectos en la producción agrícola y en la recuperación vegetal tras un incendio forestal. Por ello, en el Capítulo 3 el objetivo principal fue el de analizar la implicación de una gran variedad de factores químicos y biológicos en el desarrollo de la repelencia al agua en condiciones naturales. En concreto se estudió: la influencia de la especie vegetal, el pH, la materia orgánica, el contenido total de lípidos, la glomalina, el micelio total y el ergosterol. En este caso la textura no fue considerada como factor, ya que todas las muestras procedían de la misma área y este factor ya ha sido ampliamente estudiado. De todos los parámetros analizados, la calidad de la materia orgánica, estudiada a través del contenido total de lípidos, fue el principal factor involucrado en la aparición de la repelencia al agua. Casi todas las correlaciones encontradas entre la repelencia y el resto de parámetros, entre ellos la glomalina, fueron debidas a la relación indirecta de estos con la materia orgánica. En el caso del pino, también destacó la influencia de la actividad de los hongos. Relación que podría deberse a la composición de la materia orgánica (cantidad y tipo de compuestos hidrofóbicos concretamente), ya que estos tienen un papel muy importante en la descomposición de la materia orgánica más recalcitrante. Las conclusiones de este trabajo dieron lugar a plantearnos la siguiente cuestión ¿cuál es la relación entre la composición de la comunidad microbiológica y la repelencia al agua? Pregunta que tratamos de resolver en el Capítulo 4, donde analizamos la composición de la comunidad microbiana del suelo bajo la influencia de diferentes tipos de vegetación y persistencia de repelencia al agua. Los resultados revelaron una fuerte relación entre composición de la comunidad microbiana y la persistencia de la repelencia al agua en el suelo. La acumulación de compuestos hidrofóbicos (más difíciles de degradar) y los cambios que esto conlleva (como humedad y pH) estaría causando cambios en la composición microbiana, debido al papel de estos en la mineralización de la materia orgánica. La proporción biomasa microbiana/materia orgánica fue menor en las muestras hidrofóbicas, indicando un desequilibrio entre el aumento de microorganismos con el incremento de materia orgánica (y por tanto en compuestos hidrofóbicos). La presencia y persistencia de estos compuestos en el suelo depende en parte, de la presencia de microorganismos en el suelo capaces de degradarlos. En este sentido, las actinobacterias fueron el grupo funcional más directamente relacionado con la repelencia al agua, y por tanto en la degradación de los compuestos hidrofóbicos.

SENSITIVITY OF GLOMALIN TO FIRE EFFECTS ON SOIL AND ITS RELATIONSHIP WITH WATER REPELLENCY IN MEDITERRANEAN FORESTS SOILS

ABSTRACT

Soils are essential for life, so understanding how they work and respond to environmental disturbances is crucial to carrying out a proper maintenance and use of them. The peculiarities of the Mediterranean climate, characterized by a very hot and dry summer season, are the reason why forest fires and water use have been of special interest in the last decades in the southeast of Spain.

In this PhD thesis, both issues have been addressed through the study of a soil component, glomalin (a glycoprotein produced by arbuscular mycorrhizal fungi), which get into the ground when hyphae die and decompose. This glycoprotein is being widely studied due to its contribution to soil carbon and nitrogen storage, its role in soil structure (through the formation of aggregates), and the sequestration of potentially toxic soil elements among others. It is also known for its sensitivity to perturbations such as changes in land management. In concrete, in this PhD thesis, glomalin has been studied as a soil component that can provide information about the status of the soil after a forest fire, and as a possible factor involved in the development of soil water repellency, a phenomenon which can occur under natural conditions and which is also very common as a consequence of wildfires.

The general objective of this PhD thesis, "Sensitivity of glomalina to fire effects on soil and its relationship with water repellency in Mediterranean forest soils", is to study the effects of fires on glomalin stocks, as well as the factors involved in the appearance of water repellency and its consequences on the microbiological community in Mediterranean forest soils. The thesis is structured in four chapters with journal format. All chapters have been published as articles in international impact journals such as "Land Degradation and Development" (Chapter 1), "Science of the Total Environment" (Chapter 2), "Geoderma" (Chapter 3) and "Journal of Hydrology and Hydromechanics" (Chapter 4).

Fire is a natural ecological factor, which was a phenomenon long before human existence. In fact, fire plays an important role in the maintenance of the structure and functionality of the ecosystems. Unfortunately, in the last decades, certain human factors, such as agricultural abandonment and fire suppression policies among others, have provoked a change in the fire regime and intensity.

Soil is a basic component of the forest ecosystem and the remaining components depend on it. The immediate effect of heat and post-fire conditions cause physical, chemical and biological changes in it. Depending on certain factors and the environmental conditions, these changes can last for years. Therefore, an evaluation of soil health after a fire is essential for its proper conservation and recovery. In consequence, finding components or soil indicators able to inform about soil evolution and recovery after a wildfire is of major importance. In this regard, in Chapter 1, glomalin response to temperature was studied. To achieve this, eight different soils from Alicante province were burned under laboratory conditions at six temperatures (180, 200, 250, 300, 400 and 500°C). Soil organic matter and soil water repellency (commonly used as fire severity indicators) response to temperature was also analysed to evaluate the usefulness of glomalin as an indicator of fire severity. Soils analysed were mainly selected on the basis of soil texture; 3 sandy soils, 4 loam soils and 1 clay soil. Glomalin content response to temperature was different between soils. Response to temperature was similar between soils with similar textures. Other soil properties such as aggregate stability and organic matter content also influenced the response of glomalin content to different temperatures. However, the response of organic matter to temperature was very similar between different soils. Water repellency did not appear in any of the soils after the different treatments, except for those that were already hydrophobic in natural conditions. Soil water repellency completely disappeared at over 200°C. The combined use of different sensitive soil parameters to temperatures could provide very useful information about soil fire severity.

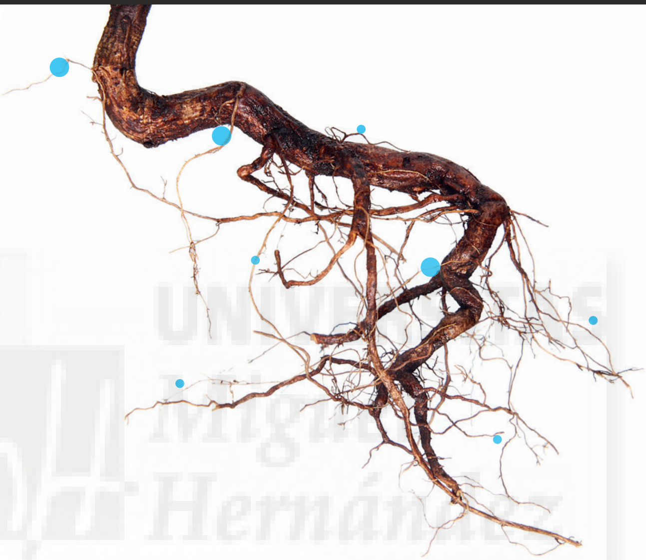
Burning experiments under laboratory conditions are needed to study the direct effects of temperature on soil properties, although field and laboratory conditions will not be exactly the same. Therefore, the next step was to analyse the response of the glomalin after a wildfire. In Chapter 2, the sensitivity of glomalin to fire effects on soil under the influence of two vegetal species (pine *vs* shrubs) was examined. Immediate effects of fire were studied in two different areas: Gorga and Gata de Gorgos (both located in Alicante province), while medium-term effects were only studied in Gorga. Different plots were installed to be monitored quarterly during a year. Glomalin content immediately after the fire only significantly changed in shrub plots in Gorga, so fire severity was higher in those plots. The results of organic matter also showed a higher fire severity in shrub plots, in this case in both study sites. The different results observed between glomalin and organic material response to fire could be due to the different response to temperature between parameters. Medium-term fire effects in glomalin content were observed in both types of plots. Glomalin contents in the control plots were variable across the

year, while burned plots glomalin content did not change during the same period of study. The lack of variability could be due to a low rate of both glomalin production and mineralization.

Furthermore, glomalin has been considered as a possible factor involved in the development of soil water repellency. The observation of this phenomenon is of special relevance in a semiarid area, like the Southeast of Spain. Soil is responsible for water storage and distribution. The presence of surface soil water repellency will limit vegetation cover growth and microbiological activity, and therefore all the factors that depend on it, such as biogeochemical cycles, which are crucial for the proper functioning of the ecosystems. Thus, it is not surprising that soil water repellency has received special attention in the last decades. Water repellency is a very complex phenomenon whose appearance can be linked to many factors such as plant species, organic matter, texture, pH, moisture, forest fires etc. It is a difficult property to study and has important environmental implications (positive and negative). Studying its causes and ecological implications is crucial to understanding the real implications for agricultural production and vegetation recovery after a wildfire. Therefore, the main objective of Chapter 3 was to find out which factors are the most relevant in the development of soil water repellency. A large quantity of chemical and biological factors were studied, in concrete, plant species influence, pH, organic matter, total lipid content, glomalin, total mycelium content and ergosterol were analysed. Texture was not considered as a factor since all samples belong to the same area and this factor has been extensively studied. The quality of soil organic matter, in concrete extractable lipid content, was the major responsible factor in soil water repellency. In general, correlations between soil water repellency persistence and other parameters, including glomalin, were related to soil organic matter content. However, fungal activity was also a significant factor in the particular case of pine samples. Fungi have a special role in the mineralization of recalcitrant compounds, so quality and quantity (amount and type of hydrophobic compounds) could be the main reasons for the correlation found. The conclusions of this study led us to ask the following question: what is the relationship between soil microbial community composition and soil water repellency persistence? Therefore, we tried to answer that question in Charpter 4, where we studied, the composition of the soil microbial community under the influence of different plant species and persistence of water repellency. The results revealed a strong relationship between the microbial community composition and persistence of the soil water repellency. Accumulation of hydrophobic compounds (more difficult to degrade) and small differences in soil properties (like moisture and pH) may be causing changes in microbial composition, due to their role in the mineralization of organic matter. The ratio microbial biomass/soil organic carbon was lower in the hydrophobic

samples. This indicates an imbalance between growth of microorganisms and organic matter content (and therefore hydrophobic compounds) input. The presence and persistence of these compounds in soil partially depends on the presence of soil microorganisms capable of degrading them. In this sense, the functional group actinobacteria were the most directly related to water repellency, and therefore in the degradation of hydrophobic compounds.

INTRODUCCIÓN



Los suelos actúan como los grandes digestores del Sistema Tierra. Son los encargados de filtrar las aguas, aportar los sedimentos, minerales y nutrientes a través de la meteorización y los ciclos biogeoquímicos. El mantenimiento de los ecosistemas terrestres depende de la salud del suelo, es decir, que éste cumpla con sus funciones correctamente. Estudiar el subsistema suelo es clave para poder entender los posibles efectos ambientales de otros elementos del ecosistema como el fuego y la distribución del agua, agentes fundamentales en muchos otros procesos como el ciclo geológico y la distribución de las plantas (Mataix-Solera y Cerdà, 2009).

El fuego elimina temporalmente la vegetación y afecta al horizonte superficial del suelo, teniendo consecuencias directas sobre los ciclos hidrológicos, en concreto en el proceso de infiltración, modificando el comportamiento hidrofílico/hidrofóbico del suelo (Cerdà y Robichaud, 2009; Doerr et al., 2000). Sin embargo, este comportamiento hidrofílico/ hidrofóbico del suelo que condiciona la infiltración del agua en el suelo, es también un fenómeno frecuentemente observado en condiciones naturales en muchos tipos de suelos (Doerr et al., 2000).

En esta tesis hemos estudiado ambas temáticas a través del estudio de un componente del suelo, la glomalina. En concreto se ha estudiado como se ve afectada por el fuego, así como la información que podría proporcionarnos acerca del mismo, y por otro como factor contribuyente a la aparición de hidrofobicidad al agua en el suelo en condiciones naturales.

Primeramente explicaremos qué es la glomalina y a continuación desarrollaremos tanto la actualidad y las consecuencias de los incendios forestales sobre los suelos, así como las características de la repelencia al agua en suelos forestales en condiciones naturales.

GLOMALINA

La glomalina es una glicoproteína producida principalmente por hongos micorrícicos arbusculares (HMA) (Wright y Upadhyaya, 1996), un tipo de hongo micorrícico caracterizado por la formación de asociaciones mutualistas obligatorias con las plantas (fig. 1). Lo que diferencia a este tipo de hongo micorrícico del resto, es la formación de hifas intracelulares y arbúsculos (donde tiene lugar el intercambio entre el hongo y la planta) en las células corticales de la raíz. En algunos casos también pueden presentar unas estructuras de reserva llamadas vesículas. Las raíces de la plantas con HMA no presentan una morfología diferente a las no micorrizadas.

Este tipo de hongo está presente en más de un 70% de todas las especies de plantas y cuenta con 8 géneros diferentes incluidos en el grupo *Glyceromicota* (Schubler et al., 2001). Los

HMA forman asociaciones con un gran número de especies de importancia en el ecosistema mediterráneo como el lentisco (*Pistacia lentiscus*), el espino negro (*Rhamnus lycoides*), el palmito (*Chamaerops humilis*), el enebro (*Juniperus oxycedrus* y *J. communis*), así como de especies arbóreas como el chopo (*Populus sp*.), las sabinas (*J. sabina, J. thurifera, J. phoenicea*), el arce (*Acer sp*.), el sauce (*Salix sp*), etc.

Otra de las características singulares de este tipo de hongos es sin duda la producción de glomalina, compuesto que se caracteriza por ser estable, insoluble en agua y altamente resistente a la degradación por el calor. Esta glicoproteína fue descrita por primera vez por Wright y Upadhyaya (1996) y debe su nombre al *phylum Glomeromycota,* ya que además de este grupo, ningún otro grupo de hongos la produce en cantidades significativas (Wright y Upadhyaya, 1996). Es producida por las células de las paredes de las hifas del hongo y se acumula en el suelo tras la muerte y descomposición de las mismas (Driver et al., 2005).

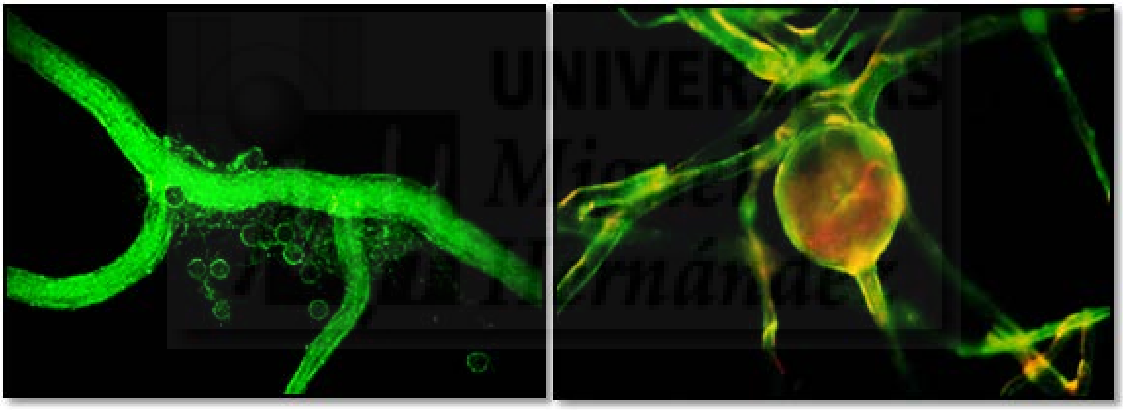


Figura 1: Imágenes de glomalina recubriendo las paredes del hongo micorrícico y de las esporas en una raíz de maíz. Fotografía: Sara Wright

Debido a que el proceso de extracción de la glomalina del suelo, no garantiza la completa eliminación de otras proteínas resistentes al calor, la glomalina cuantificada y extraída del suelo, operacionalmente se denomina “Glomalin Related Soil Protein (GRSP)” (distinguiendo a su vez entre “total y “easily extractable”; Tabla 1), siendo el término Glomalina, el nombre del producto codificado por el gen putativo de los HMA (Rillig, 2004; Tabla 1). Sin embargo, hay estudios que han demostrado que la glomalina extraída del suelo es muy similar a la glomalina de las hifas del hongo (Rillig et al., 2001; Wright et al., 1999).

Tabla 1: Terminologías actuales para glomalina y sus definiciones (Extraído de Singh et al., 2013).

Término	Descripción
Glomalina	Gen putativo todavía por identificar producido por los HMA
Glomalin related soil protein (GRSP)	Fracción de glomalina extraída de suelo que posiblemente contiene otras proteínas resistentes al calor
Total glomalin related soil protein (T-GRSP)	Fracción total de glomalina en el suelo extraída mediante buffer 50 nM de citrato (pH 8) y ciclos repetidos de autoclave a 121°C durante 60’/ciclo.
Easily extractable glomalin related soil protein (EE-GRSP)	Corresponde a la fracción de glomalina más recientemente depositada en el suelo. Extraída mediante buffer 20 nM de citrato (pH 7) y un solo ciclo de autoclave a 121°C durante 30’.

Estructura y composición molecular de la glomalina

La molécula de la glomalina es definida como poco corriente, compleja y recalcitrante (Singh et al., 2013), características que unidas a la posible presencia en los extractos de otros compuestos del suelo hacen que la determinación de su estructura bioquímica a través de extractos del suelo sea complicada. De hecho, no existe un gran número de trabajos publicados en este campo. La glomalina es definida como una glicoproteína N-ligada (Koide y Mosse, 2004), cuya composición química puede variar de: 3 a 5% de nitrógeno, 36 a 59% de carbono (Lovelock et al., 2004a; Schindler et al., 2007), 4 a 6% hidrógeno, 33 a 49% de oxígeno, 0.03 a 0.1% de fósforo (Schindler et al., 2007) y por último de 0.8 a 8.8% de hierro, siendo este el responsable del color rojizo por el que se caracterizan los extractos de glomalina (Wright y Upadhyaya, 1998). En cuanto a su estructura bioquímica, Schindler et al. (2007) encontraron un alto contenido en compuestos aromáticos (Koide y Mosse, 2004) y carboxílicos (Keiblinger et al., 2012), y bajo en alifáticos e hidratos de carbono (Lovelock et al., 2004a).

Por otro lado, los ratios de C/N revelan que la glomalina extraída del suelo tiene contenidos en N inferiores a las proteínas pero superiores a los ácidos húmicos, lo que sugiere que, la glomalina extraída del suelo, es un compuesto mixto de sustancias proteicas y húmicas (Koide y Peoples, 2013).

Extracción y cuantificación de la glomalina del suelo (GRSP)

Existen cuatro medidas diferentes de GRSP, que son definidas según el método de detección y el proceso de extracción realizado (Treseder y Turner, 2007). La GRSP fácilmente extraíble corresponde a la fracción de glomalina extraída durante el primer ciclo de extracción, cuando los suelos son autoclavados a 121°C durante 30 minutos, usando como solución extractante citrato de sodio (20mM) a pH 7. Para la extracción de la GRSP total (“*total GRSP*”), sin embargo, se realizan ciclos sucesivos de extracción hasta que el extracto pierde el color anaranjado típico (Wright y Upadhyaya, 1998). Los ciclos de extracción son más largos (60 min) y con una solución de citrato de sodio más concentrada (50mM) y alcalina (pH 8) (Treseder y Turner, 2007).

La glomalina fue identificada por primera vez gracias al desarrollo de la técnica inmunoenzimática (ELISA), en la que se utiliza un anticuerpo monoclonal (MAb32B11) específico para este compuesto de los HAM (Rillig y Steinberg, 2002; Rosier et al., 2006). Debido a que muchos laboratorios no disponen de la capacidad para llevar a cabo la medición de GRSP mediante el método inmunoreactivo, es muy común encontrar en la literatura la medida de glomalina mediante el método colorimétrico del reactivo Bradford (Rosier et al., 2006). Es evidente que este último método es menos específico que el inmunoreactivo, por ello, algunos trabajos señalan que el método Bradford tiende a sobreestimar las cantidades de GRSP, sin embargo las correlaciones existentes entre ambos métodos, son indicativos de la eficacia del método Bradford para la determinación relativa de GRSP (Koide y Peoples, 2013; Rosier et al., 2006; Tabla 2).

Tabla 2: Métodos de extracción de la glomalina del suelo.

Técnica	Descripción	Método de cuantificación
Bradford-reactive soil protein (BRSP)	GRSP cuantificada mediante el método Bradford por colorimetría. Tiene la desventaja de que puede sobreestimar los resultados ya que puede medir otro tipo de proteínas del suelo.	**Total Bradford related soil protein (T-BRSP)**: mide el contenido total de glomalina mediante método Bradford. **Easily extractable Bradford related soil protein (EE-BRSP)**: contenido de la glomalina fácilmente extraíble mediante método Bradford.
Inmuno Reactive Soil Protein (IRSP)	GRSP cuantificada mediante técnica inmunoreactiva (ELISA) usando un anticuerpo monoclonal (MAb32B11) específico para este compuesto de los HAM. Tiende a dar cantidades inferiores de glomalina a las existentes debido a la interferencia de otras proteinas.	**Total Inmuno Reactive Soil Protein (T-IRSP)**: mide el contenido total de glomalina mediante técnica inmunoreactiva. **Easily extractable Inmuno Reactive Soil Protein (EE-IRSP)**: contenido de la glomalina fácilmente extraíble mediante técnica inmunoreactiva específica.

Distribución de los stocks de glomalina, producción y descomposición

La asociación entre HMA y plantas es probablemente una de las más importantes en la Tierra, siendo el nexo de unión entre las raíces y el sistema suelo (Koide y Mosse, 2004). Como ya decíamos, cerca del 70% de las familias de plantas forman asociaciones mutualistas con los HMA y están presentes en grandes cantidades en la mayor parte de los biomas terrestres (Treseder y Turner, 2007). Por lo que no es de extrañar que la glomalina haya sido encontrada en suelos de todo tipo de biomas: bosques (boreales, templados), desiertos, praderas, selvas tropicales y diferentes tipos de manejos agrícolas e incluso en zonas contaminadas (Tabla 3). GRSP ha sido encontrada también en llanuras aluviales y lechos de los ríos (Singh et al., 2013).

Tabla 3: Presencia de glomalina en diferentes biomas terrestres.

Tipo ecosistema	Localización	Referencias
Agrícola	Chile	Borie et al. (2000)
	Italia	Bedini et al. (2007)
	Sud África	Preger et al. (2007)
Bosque Boreal	Alaska (EEUU)	Treseder et al. (2004)
Bosque Templado	Estados Unidos	Nichols y Wright (2005)
Semidesertico	España	Rillig et al. (2003a)
Estepas semideserticas	Mongolia	Wang et al. (2014)
Desierto	California (EEUU)	Clark et al. (2009)
Pradera	Montana (EEUU)	Lutgen et al. (2003)
Selva Tropical	Brasil	Bonfim et al. (2013)
	Hawai	Rillig et al. (2001)

Sin embargo, esas concentraciones varían mucho de unos biomas a otros, hecho que puede ser atribuido a las diferencias ambientales que influyen directamente en la producción primaria neta y en la abundancia de los HMA en cada uno de los biomas (Treseder y Turner, 2007).

La glomalina aparece como extraíble del suelo tras la muerte y descomposición de las hifas de los HMA, por tanto, los stocks de GRSP estarán determinados por su producción y descomposición, y las condiciones ambientales que pueden afectar a los dos flujos independientemente (Rillig, 2004). Más específicamente, la producción de glomalina depende de la presencia y producción de los HMA, siendo por tanto, controlada por los factores que influyen a estos. Entre ellos destacan: la planta hospedadora, la producción del hongo y la tasa de descomposición de las hifas (Treseder y Turner, 2007). A nivel de ecosistema, los stocks de GRSP también pueden verse afectados por las condiciones del suelo (disponibilidad de nutrientes, agua, agregados y manejo del suelo) y diferentes parámetros ambientales (precipitación y temperatura; Rillig, 2004; fig. 2).

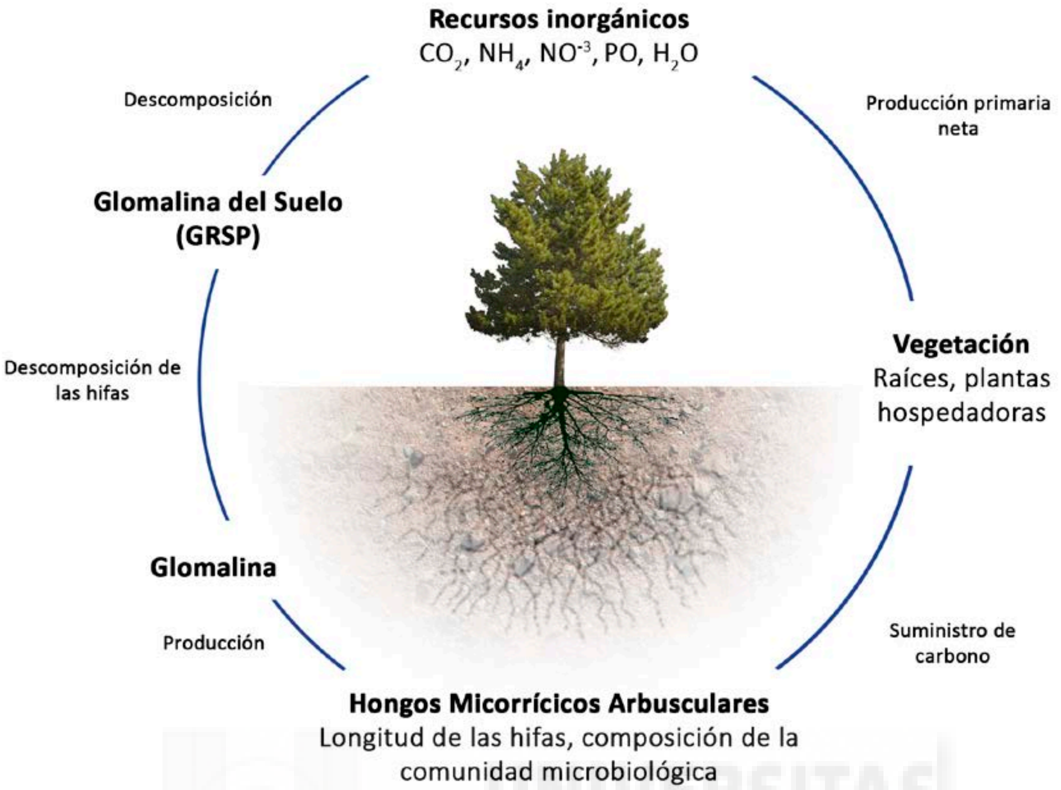


Figura 2: Ciclo de la glomalina en el suelo. Modificado a partir de Treseder y Turner (2007).

Factores que afectan a la abundancia de los stocks de GRSP

- **Planta hospedadora**

Puede decirse que las plantas son los principales determinantes en la producción de glomalina. Cada planta destina al hongo diferentes porcentajes de carbono, y éste en parte será utilizado por el HMA para la producción de glomalina. De hecho, se han observado efectos positivos en la producción de glomalina que han sido directamente relacionadas con la tasa de crecimiento, el estatus nutritivo de las plantas (Violi et al., 2008), la biomasa y la calidad y cantidad de la materia orgánica (Wilson et al., 2009). Lo que puede atribuirse a la dependencia de los HMA a la abundancia de las plantas hospedadoras y su productividad primaria neta (Treseder y Turner, 2007).

- **Abundancia y especie de HMA**

Por otro lado, cabría esperar que las tasas de acumulación de glomalina en el suelo fueran dependientes de la cantidad de hifas, la cantidad de glomalina por hifas y la tasa de reposición de las mismas. Sin embargo, esta relación no es tan directa, y estos parámetros no están siempre correlacionados. Los datos acerca de la relación entre la longitud de la hifas y la cantidad de glomalina son inconsistentes, se encuentran tanto trabajos donde estos parámetros se correlacionan (Treseder et al., 2004), así como en los que no (Borie et al., 2000; Lovelock et al., 2004b; Lozano et al., 2013). Esta ausencia de correlación puede deberse a varios factores: diferencias en el contenido de glomalina entre hifas de diferente diámetro, ya que éste no se tiene en cuenta en las técnicas normalmente utilizadas, o diferencias en las tasas de descomposición entre las hifas y glomalina. Steinberg y Rillig (2003) observaron que la duración de la glomalina en el suelo es mucho mayor que la de las hifas. También habría que tener en cuenta, que no todas las especies invierten lo mismo en la producción de glomalina. Diferentes estudios indican que los géneros *Giaspora* y *Acaulospor*a invierten más en la producción de glomalina que el género *Glomus* (Lovelock et al., 2004b; Wright y Upadhyaya, 1999), cuya abundancia es mayor en suelos minerales (Treseder y Turner, 2007).

- **Condiciones ambientales y edáficas**

Las condiciones ambientales influyen en ambos flujos: producción y descomposición. Las plantas tienden a invertir más en los HMA cuando los recursos hídricos son bajos (Auge, 2001). No existen muchos trabajos acerca de la estacionalidad de los stocks de GRSP, la baja tasa de descomposición hace pensar que los stocks no tienen por qué ser muy variables a priori. Sin embargo, a pesar de ello, Lutgen et al. (2003) observaron estacionalidad, aunque leve en un estudio llevado a cabo durante 7 meses en un ecosistema de pradera. Serían necesarios más trabajos sobre la estacionalidad de la GRSP durante períodos más largos y en diferentes ecosistemas.

Las condiciones edáficas pueden alterar las tasas de descomposición de GRSP a través de factores como la disponibilidad de nutrientes (que puede incrementar la actividad microbiana (Lovelock et al., 2004b) y la textura. Un mayor contenido en arcilla proporcionará una mayor protección física a la degradación de la glomalina (Nichols y Wright, 2005) debido a una mayor agregación de partículas y la consiguiente protección en su interior.

Funciones de la glomalina en el suelo y su significado ecológico

El papel de glomalina en el ecosistema es todavía desconocido. Desde su descubrimiento, son varias las hipótesis que han aparecido como consecuencia de su relación con diferentes parámetros del suelo: como son la hidrofobicidad o repelencia al agua (SWR, Soil Water Repellency), la agregación de partículas y la estabilidad de los agregados, el contenido de C, N, etc. Se ha llegado a relacionar la GRSP con una clase de proteínas ricas en cisteína (conocidas como “hydrophobins”), presentes en los hongos filamentosos y conocidas por su naturaleza hidrófoba, estableciendo por tanto una correlación entre GRSP, SWR y la formación y estabilidad de agregados (Rillig, 2005). Sin embargo, estudios más recientes acerca de su secuencia genética y su localización en las hifas, ha hecho que algunos autores apunten más hacia una función fisiológica del hongo. La estructura de la glomalina ha sido relacionada con la de las proteínas de shock térmico hsp60 (Gadkar y Rillig, 2006), proteínas que se manifiestan en condiciones de estrés para proteger a las células de daños fisiológicos como el calor, cambios de pH o condiciones oxidantes. Se ha mencionado incluso que, debido a su localización en las hifas del hongo podría estar relacionada con la disminución de la palatabilidad del micelio frente a depredadores, relegando su relación con los parámetros ambientales a efectos ambientales secundarios, mas que a funciones de la glicoproteína (Purin y Rillig, 2007).

El papel de la GRSP en la agregación del suelo

Desde su descubrimiento hasta la actualidad, la GRSP ha sido considerada como un compuesto que favorece la unión de las partículas del suelo, dando lugar por tanto, a la formación de agregados (Bedini et al., 2009; Wu et al., 2014a; Wu et al., 2014b; fig. 3). Son muchos los trabajos los que han encontrado una relación logarítmica significativa (Wilson et al., 2009; Wright y Anderson, 2000; Wright y Upadhyaya, 1998) y muy pocos los que han encontrado una relación sigmoidal (Harner et al., 2004; Spohn y Giani, 2010) o ausencia de la misma (Batten et al., 2005; Rillig et al., 2003a). Los resultados del modelo estadístico utilizado en Rillig et al. (2002) mostraron que el efecto directo de la GRSP en la estabilidad de agregados fue mayor que el de las hifas, aunque similar al de las raíces de las plantas. En un trabajo posterior, este mismo autor (Rillig, 2004), sugiere que esta relación es más evidente en aquellos suelos donde la materia orgánica es el principal agente en la formación de agregados.

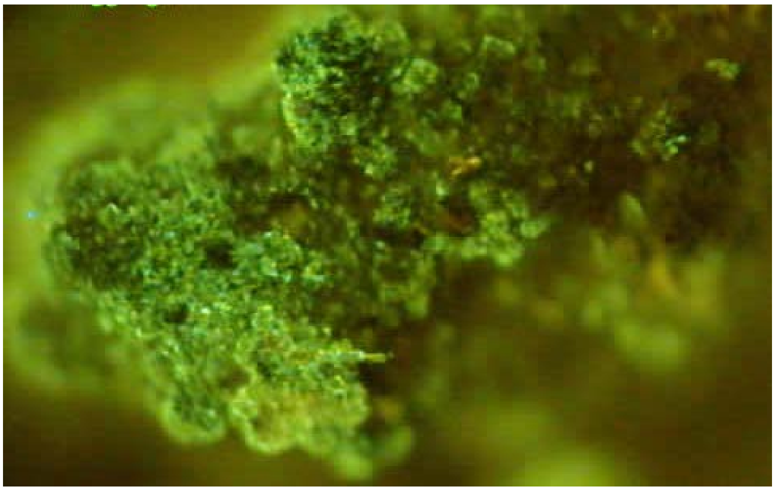


Figura 3: Glomalina recubriendo agregados del suelo (Wright y Upadhyaya, 1996).

Debido a que los agregados del suelo intervienen en el almacenamiento de agua, nutrientes, y el intercambio de gases (Rillig y Mummey, 2006), la relación de GRSP con la formación de agregados podría tener un significado ecológico a través de la modificación del entorno (Rillig y Steinberg, 2002), incrementando así la productividad a través de la mejora de la aireación del suelo, el drenaje y la actividad microbiológica (Lovelock et al., 2004a), reduciendo a su vez la degradación de la materia orgánica, que quedaría protegida en el interior de los agregados (Wright et al., 2000).

El papel de la GRSP como reserva de C y N del suelo

Las moléculas de GRSP tienen un alto contenido en átomos de C y N, siendo importante fuente de estos elementos para el suelo (Fokom et al., 2012; Nichols y Wright, 2006), donde puede llegar a representar un alto porcentaje de su contenido total acumulado (Nichols y Wright, 2006). Este porcentaje es variable y depende del tipo de suelo. Se han registrado cantidades que van desde un 5% del C y N del suelo en suelos tropicales (Lovelock et al., 2004a; Rillig et al., 2001), hasta un 25% y un 52% de C distinguiendo entre suelos minerales y orgánicos respectivamente (Schindler et al., 2007).

La contribución de la GRSP en la reserva de N y C del suelo es debida principalmente a dos factores: 1) GRSP es un compuesto bastante resistente a la degradación, por lo que tiende a acumularse en el suelo (Halvorson y Gonzalez, 2006; Rillig et al., 2001), y 2) reduciendo la tasa de mineralización de la materia orgánica a través de su papel en la formación de agregados (Wright et al., 2000). Wilson et al. (2009) observaron una reducción del contenido de C y N del suelo debido a la supresión de los HMA, hecho que relacionaron con un significativo descenso de las hifas y las concentraciones de GRSP. Este descenso podría atribuirse al aumento de la mineralización de estos elementos tras la pérdida de agregados, donde permanecían protegidos.

El papel de la GRSP en el secuestro de metales pesados

Los HMA son capaces de tolerar altas concentraciones de metales potencialmente tóxicos (González-Chavez et al., 2002), y la glomalina es en parte responsable de este mecanismo (González-Chavez et al., 2004). En los últimos años ha habido un creciente interés en el estudio de GRSP como potencial secuestrador de metales pesados en el suelo (Seguel et al., 2013; Singh et al., 2013). GRSP es capaz de unirse y secuestrar en cantidades considerables metales pesados como: Cu (Cornejo et al., 2008), Al (Aguilera et al., 2011), Cr (Gil-Cardeza et al., 2014) Pb y Zn (Vodnik et al., 2008a), mitigando las condiciones de stress a través de la estabilización de dichos elementos en el suelo. Aguilera et al. (2011) mostraron que la glomalina es capaz de secuestrar Al en el interior de la molécula, creando un complejo que, teniendo en cuenta la resistencia a la degradación de la GRSP (Rillig et al., 2001), se acumulará en el suelo de manera estable durante mucho tiempo. El incremento de esta proteína ante situaciones de stress (Vodnik et al., 2008a) podría estar relacionado con mecanismos de los propios hongos para la reducción de la toxicidad en los sistemas de raíces micorrizadas (Seguel et al., 2013).

El papel de la glomalina en la reducción de los gases de efecto invernadero

Las emisiones de CO_2 procedentes del suelo a la atmósfera son en su mayor parte atribuidas a la destrucción de la estructura del suelo debido a malas prácticas agrícolas (Janzen, 2004). Por ello, todos aquellos parámetros que promueven la formación de agregados, reducirán las pérdidas de C. Son muchos los trabajos donde se ha demostrado que el laboreo excesivo disminuye las concentraciones de GRSP y la estabilidad de agregados al agua respecto a zonas forestales o cultivos abandonados (Fokom et al., 2012; Hontoria et al., 2009; Roldán et al., 2007). Por otro lado, se ha comprobado que ante incrementos de CO_2 atmosférico, las plantas aumentan las cantidad de C que transfieren a los HMA, traduciéndose en un incremento de la producción de glomalina, que actuaría como secuestrador y fijador de C a largo plazo en el suelo (Vodnik et al., 2008b). Del mismo modo, la GRSP puede contribuir de manera indirecta a la reducción de las emisiones de N_2O a la atmósfera a través de los ciclos de nitrificación y desnitrificación, disminuyendo así las pérdidas de N del suelo (Singh et al., 2013).

INCENDIOS FORESTALES

El fuego es un factor ecológico natural en los ecosistemas terrestres, siendo un factor primordial en el mantenimiento de la estructura y funcionamiento en muchos de ellos (Pausas et al., 2008). Prueba de ello es el ecosistema Mediterráneo, caracterizado por la presencia de una vegetación que ha desarrollado numerosas adaptaciones al paso del fuego (Trabaud y Lepart, 1980). En la cuenca mediterránea el fuego es uno de los principales factores que determinan el paisaje forestal. Paisajes que han sido influenciados por el ser humano, que ha modificado el territorio para su uso y aprovechamiento durante miles de años (McNeil, 1992), pudiendo afirmar que en nuestro territorio no existen ecosistemas que no hayan estado o estén de una u otra manera influenciados por el hombre. El fuego ha sido utilizado como herramienta de gestión del territorio desde tiempos prehistóricos, fragmentando el paisaje (Wrangham et al., 1999), causando una heterogeneidad espacial en los ecosistemas entre cuyos impactos se encuentra una influencia determinante en el régimen natural de los incendios forestales.

Los efectos del fuego en los ecosistemas mediterráneos son variables y dependen de la intensidad, el área afectada y la recurrencia de los mismos (Broncano y Retana, 2004; Eugenio y Lloret, 2004; Pausas et al., 2003; Tsitsoni, 1997).

En las últimas décadas se ha visto modificado el régimen y las características de los incendios forestales, siendo ahora algunos de ellos mucho más extensos e intensos, lo que ha hecho a su vez al fuego un elemento de interés mediático. Las políticas de lucha contra los incendios e intentos de supresión del fuego del medio, el éxodo rural y por tanto el abandono de los terrenos de cultivo (Naredo, 2004), son algunos de los factores que están detrás del problema.

Factores que han provocado que grandes incendios con consecuencias ambientales, económicas y sociales devastadoras, sean una realidad recurrente, como así lo demuestran los datos de los últimos años (fig. 4).

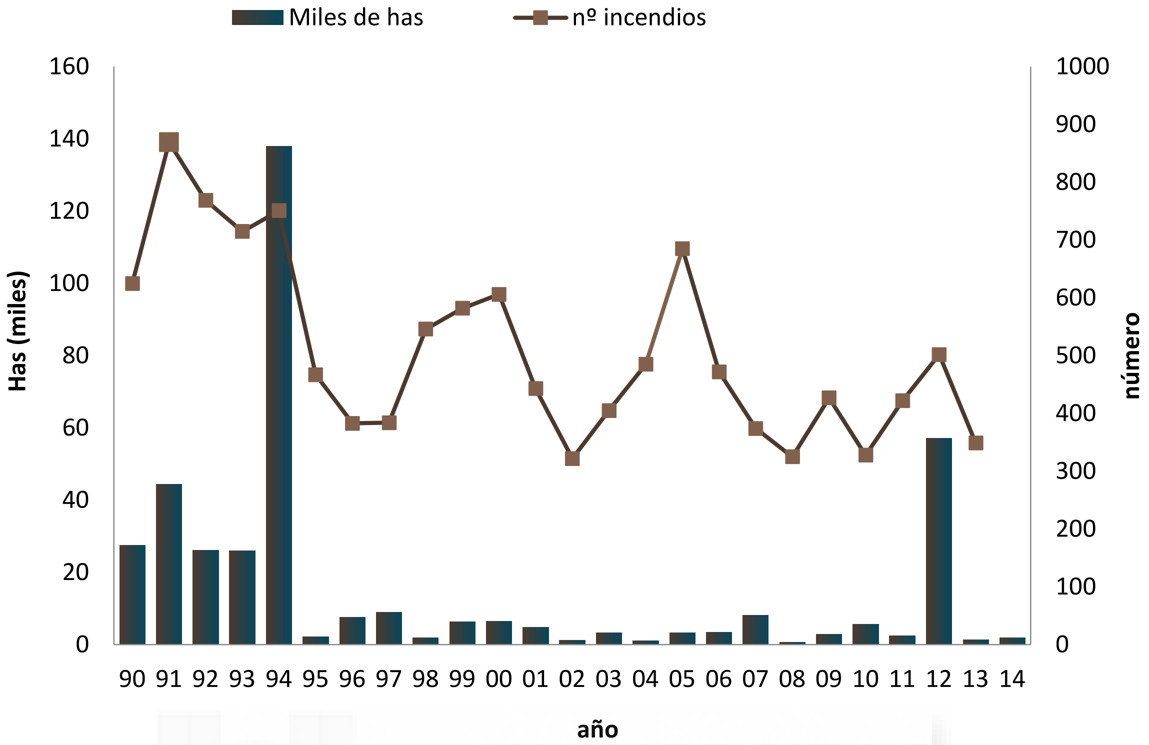


Figura 4: Número de incendios y superficie quemada (miles ha) por año en la Comunidad Valenciana. Fuente: Elaboración propia a partir de datos de la "Generalitat Valenciana, Consellería d'infraestructures, Territori i Medi Ambient".

En el año 2014 se quemaron alrededor de unas 2.000 hectáreas de bosques a lo largo de la Comunidad Valenciana, pero es sin duda el 2012 el más llamativo de la última década, no solo por el número de hectáreas quemadas, sino porque tan sólo dos incendios forestales (en las localidades de Cortes de Pallás y Andilla) abarcaron el 83% (alrededor de 50000 ha.) de la superficie quemada durante ese año.

Severidad e intensidad del fuego

Los incendios forestales suponen una alteración de todos los elementos del ecosistema; consumen parcial o totalmente la materia vegetal, alteran las propiedades del suelo, afectan a la micro y la macrofauna, alteran los procesos hidrológicos, geomorfológicos y la calidad de las aguas (Mataix-Solera y Guerrero, 2007). Alteración cuya magnitud variará según la intensidad y la severidad del fuego, parámetros que nos aportan información sobre el propio fuego, así como de sus consecuencias en las zonas afectadas por éste. Cuando hablamos de intensidad nos referimos a la velocidad a la que se libera la energía en el frente de la llama y suele expresarse como kWm-1 (fig. 5 a y c). Es un parámetro que depende de factores tales como: cantidad y tipo de combustible, continuidad de éste, su humedad, la cual dependerá de las condiciones meteorológicas en el momento de la propagación y de los días previos, etc. Por otro lado, la severidad del fuego es el grado de afectación sobre determinados elementos como son el suelo, la vegetación y el ecosistema en general (fig. 5 b y d).

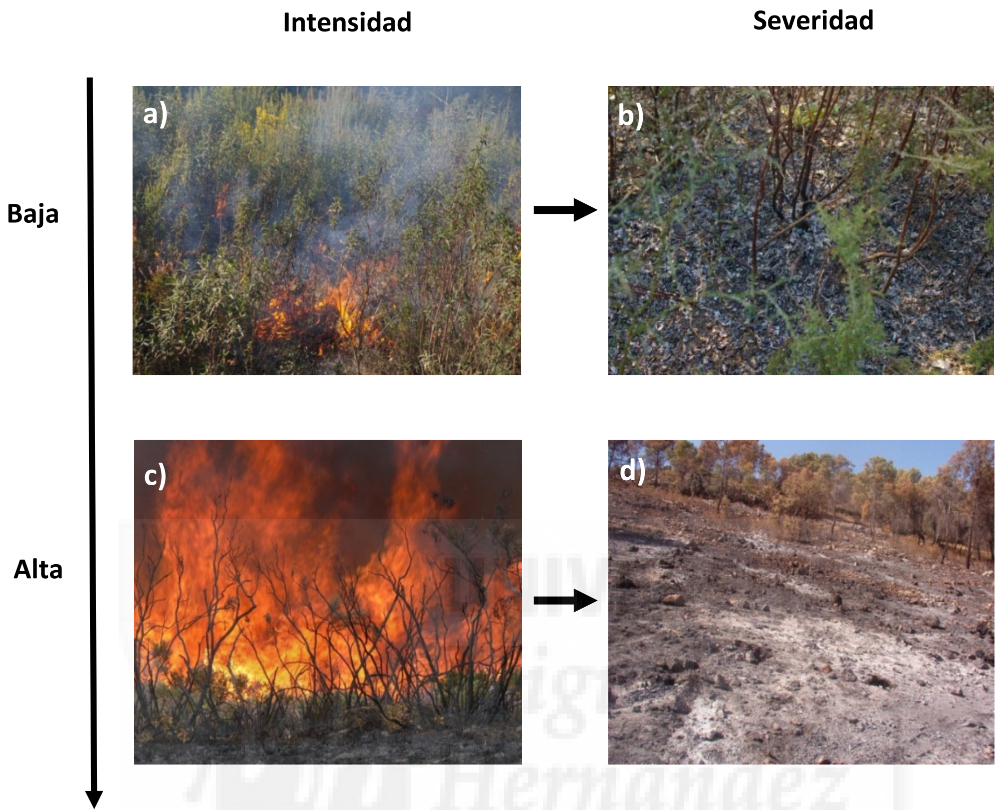


Figura 5: Diferenciación en imágenes de un fuego de baja intensidad, (a) donde las llamas son de poca altura y el grado de afectación en el suelo será poco severo (b), ya que se conservan las ramas y hojas de monte bajo y donde entre las cenizas de color negruzco se diferencia la morfología de las hojas quemadas en el suelo; y un fuego de alta intensidad (c) donde las llamas alcanzan gran altura y su grado de afectación es altamente severo (d) sobre el suelo, ya que toda la vegetación es arrasada, y donde las cenizas que quedan son de color blanquecino, sin diferenciarse ninguna morfología entre ellas. Fotografías *a*, *b* y *d*: J. Mataix-Solera, *c:* V. Arcenegui. (Extraída de Lozano y Jiménez Pinilla, 2013).

La severidad depende entre otros factores de la intensidad del fuego, así como del comportamiento del fuego, del tipo de vegetación, el tipo de suelo etc. (Keeley, 2009; Moreno y Oechel, 1989). En la Tabla 4 se describen las cinco categorías de severidad del fuego descritas por Ryan y Noste (1985). A igual severidad e intensidad no todos los suelos responderán de la misma manera, dependerá de la vulnerabilidad de los mismos y de muchos otros factores como la capacidad de resistencia y resiliencia del ecosistema.

Tabla 4: Cambios en la vegetación y la materia orgánica del suelo según la severidad del fuego (Ryan y Noste, 1985). Extraída de Keeley (2009).

SEVERIDAD DEL FUEGO	DESCRIPCIÓN
No quemado	Suelo y vegetación no alterada por los efectos del fuego
Chamuscado	Plantas no quemadas pero con muestra de pérdida de hojas por efecto del calor radiante
Suave	Copas de árboles con hojas verdes pero tallos chamuscados Hojarasca, musgos y hierbas carbonizadas o consumidas Horizontes orgánicos de los suelos intactos o parcialmente afectados sólo en los primeros mm de profundidad. Fig. 5 (b)
Moderada o severa	Árboles con parte de las copas muerta, pero hojas no consumidas Sotobosque carbonizado o consumido Ramas finas muertas en superficie del suelo y troncos carbonizados Horizontes orgánicos del suelo casi completamente consumidos
Muy severa	Copas de árboles muertas y hojas consumidas Hojarasca de todos los tamaños y horizontes orgánicos de suelo completamente consumidos Deposición de cenizas blancas y materia orgánica carbonizada a varios cm de profundidad. Fig. 5 (d)

Son muchas las técnicas que han sido utilizadas por diferentes autores para la clasificación de la severidad. Entre ellas destacan: la técnica basada en el grosor de las ramas mas finas que han quedado tras el incendio, siendo de mayor severidad cuanto mayor es el diámetro (Moreno y Oechel, 1989), el color de las cenizas y los restos de la hojarasca (Chandler et al., 1983; Wells et al., 1979), medidas de profundidad de quemado y combustión de la materia orgánica (DeBano et al., 1998; Perez y Moreno, 1998), la presencia de repelencia al agua (Doerr et al., 2004). La teledetección también es muy utilizada, permite obtener mapas de severidad (Hammill y Bradstock, 2006) aunque con el inconveniente de que generalmente no informan de la severidad sobre el suelo, sino que están basados en cambios producidos sobre la cubierta vegetal y la exposición del suelo (Lewis et al., 2006).

Efectos de los incendios forestales sobre los suelos

El suelo es un componente básico del ecosistema terrestre del que depende el reciclaje de la materia (Mataix-Solera y Guerrero, 2007). El efecto del calor en el suelo puede generar modificaciones directas que afectan a las propiedades físicas, químicas (Úbeda et al., 2009) y biológicas (Bárcenas-Moreno et al., 2011 a, b), así como cambios microclimáticos, siendo a su vez más vulnerable a la erosión (Certini, 2005). Estas alteraciones pueden retrasar el crecimiento posterior de las comunidades vegetales, generando una mayor exposición del suelo frente a los agentes erosivos. Por ello, su conservación tras un incendio es de carácter primordial, ya que sin un suelo fértil el ecosistema no podrá recuperarse adecuadamente.

Son muchas las propiedades del suelo que pueden verse afectadas tras el paso del fuego; bien de manera directa (efecto del calentamiento) o indirecta (alteraciones en las condiciones edafoclimáticas; pérdida de vegetación y recubrimiento por cenizas; Neary et al., 1999). La extensión y duración de esos efectos depende de la severidad del fuego, que en parte está controlada por muchos otros factores que afectan al proceso de combustión como cantidad, naturaleza, humedad del combustible (vivo y muerto), el viento, la humedad ambiental y la topografía (Certini, 2005). La duración del fuego es probablemente el componente de la severidad que más afectará el ecosistema edáfico (Certini, 2005). Tras el paso del fuego, la duración de los cambios producidos en las propiedades del suelo son muy variables, pudiendo ser permanentes o desaparecer a corto, medio o largo plazo (Certini, 2005). Estas alteraciones se traducirán en cambios en la química atmosférica al alterar el intercambio gaseoso, alteraciones en la calidad y cantidad de las aguas que fluyen a través de los suelos y con ello en los procesos y formas terrestres.

Los suelos, por tanto, son clave para entender los ciclos biogeoquímicos del planeta. Cuando los suelos sufren un incendio, el equilibrio conseguido durante años en los ciclos biogeoquímicos se rompe y los ecosistemas entran en una fase de cambio (Cerdà y Mataix-Solera, 2009).

La influencia del fuego en los ecosistemas terrestres se produce a corto plazo por la eliminación y modificación de la cubierta vegetal. Pero a largo plazo son los suelos quienes van a transferir el impacto del fuego a los ecosistemas. El fuego modifica la formación de los suelos al modificar el ciclo de los nutrientes (Raison, 1979), sus propiedades físicas y químicas (Úbeda et al., 2009) y los procesos microbiológicos (Mataix-Solera et al., 2009).

Efectos en las propiedades del suelo

El efecto más evidente tras el paso de un fuego es el cambio de color del mismo. Tras el incendio, el suelo es recubierto por cenizas, cuyo color y composición variará dependiendo de las temperaturas alcanzadas en los procesos de combustión y la vegetación propia de la zona incendiada (Pereira et al., 2010; Úbeda et al., 2009; fig. 6). El suelo se ennegrece cuando las temperaturas alcanzadas varían entre los 100 y 250°C, tomando un color más rojizo a temperaturas superiores debido a la formación de óxidos de hierro (Certini, 2005). El cambio de color del suelo, la eliminación de la vegetación y las cenizas provocan aumentos de temperatura y mayores oscilaciones térmicas diarias (Mataix-Solera et al., 1999).

Las cenizas también serán las responsables de los cambios producidos tanto en el pH, como en la conductividad eléctrica debido a la solubilización de compuestos contenidos en las mismas, si bien estos cambios se dan en mayor magnitud en incendios de alta intensidad (Arocena y Opio, 2003). Cambios que tienden a ser temporales y desaparecer con la erosión de las mismas (Mataix-Solera, 1999). Las cenizas también provocarán cambios similares (pH, conductividad eléctrica) en las aguas de escorrentía (Badía y Martí, 2003; Bodí, 2012). Los incendios de gran intensidad pueden provocar fusiones térmicas en partículas de tamaño arcilla, incrementado así el tamaño de las partículas, aumentando por tanto el porcentaje de limos (Úbeda et al., 1990) y arenas (Cerdà et al., 1995). En cuanto a la estabilidad de agregados, los cambios notables aparecerán a severidades moderadas y altas, y serán dependientes de las propiedades iniciales del fuego. Podremos encontrar aumento de la estabilidad en suelos ricos en arcillas y carbonato cálcico, cuya fusión a altas temperaturas puedes endurecer los agregados o disminución de los mismos con la disminución de la materia orgánica, cuando ésta es el principal agente cementante (Mataix-Solera et al., 2011).

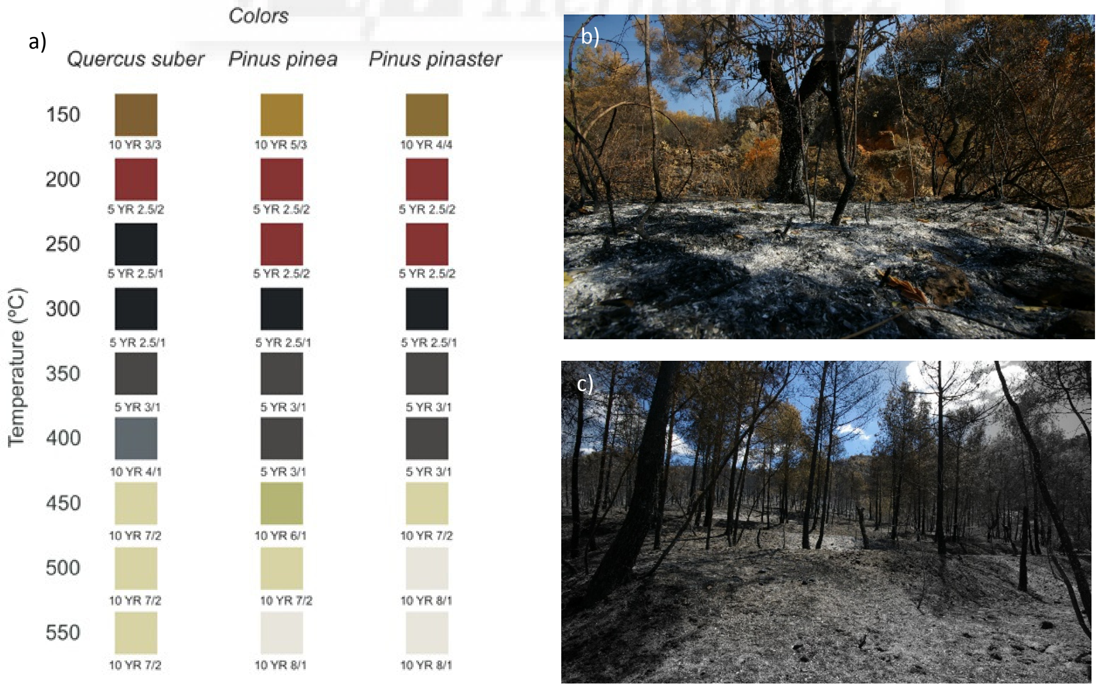


Figura 6: a) Cambio de color de las cenizas según la temperatura de combustión procedentes de *Quercus suber*, *Pinus Pinea* y *Pinus pinaster* (Extraída de Pereira y Bodí, 2013); b y c) cenizas en el suelo tras un incendio forestal. Fotografías de Jorge Mataix-Solera.

Otro de los efectos más evidentes del fuego en las propiedades del suelo es el que se produce sobre la materia orgánica. El fuego suele producir un descenso en el contenido de carbono orgánico y nitrógeno total (Marcos et al., 2007), aunque en incendios de baja intensidad y severidad puede producirse un aumento debido a la acumulación de materia vegetal semipirolizada en la superficie (Fernández et al., 2007; Mataix-Solera et al., 2002; Notario Del Pino et al., 2007). Dependiendo de la severidad del fuego, el efecto puede ir desde una ligera destilación (volatilización de los componentes más ligeros), hasta la total desaparición de la misma (Simard et al., 2001). Cuantitativamente, la combustión de la materia orgánica por el fuego comienza entre los 200 y 250°C, hasta desaparecer casi por completo cuando se alcanzan temperaturas en torno a los 460°C (Giovannini et al., 1988). El fuego tiene también efectos sobre la calidad de la materia orgánica. A nivel cualitativo se ha observado que a mayor temperatura, la materia orgánica sufre más cambios que la hacen más resistente a la descomposición biológica, lo que se denomina humus piromórfico (González-Vila et al., 2009), aumentando así la relación C/N con la temperatura. Los suelos afectados por incendios tienen un menor contenido en materia orgánica libre, ácidos fúlvicos con menor grado de polimerización y un mayor contenido de ácidos húmicos, así como una mayor proporción en humina (Almendros et al., 1984). Los cambios en el carbono orgánico hacia formas más reclacitrantes van a producir cambios en los recursos hídricos y en los cationes disponibles. Las cenizas van a aportar gran cantidad de nutrientes al suelo como fósforo (P) (Raison, 1979), Ca^{+2}, Mg^{+2}, K^{+}, Na^{+} entre otros. Sin embargo, a pesar del enriquecimiento del suelo, muchos de estos no podrán ser retenidos (Carballas, 1997). Tras el paso del fuego los componentes externos de las moléculas de ácidos húmicos y los complejos arcillo-húmicos son destruidos, disminuyendo drásticamente el contenido de nitrógeno y la capacidad de intercambio catiónico de las fracciones húmicas.

Una de las propiedades del suelo íntimamente relacionada con el fuego y que ha adquirido gran relevancia en la última década, es la hidrofobicidad o repelencia al agua del suelo (SWR; fig. 7). De hecho, la presencia de SWR tras un incendio ha sido usada como indicador de severidad post-incendio por la USDA en EEUU (USDA, 2000; Jain et al., 2012). Sin embargo, el efecto del fuego sobre esta propiedad es muy variable, dependiendo del estado natural del suelo previo al incendio y de la intensidad del mismo.

Figura 7: Presencia de repelencia al agua en el suelo tras un incendio forestal. Fotografía de Ana Mateu.

La aparición de esta propiedad tras un incendio puede tener consecuencias muy importantes que afectan a la generación de la escorrentía y por tanto al aumento de la erosión de los suelos. El efecto del fuego sobre la hidrofobicidad del suelo será descrito más en detalle en el apartado sobre hidrofobicidad.

Efectos del fuego en las comunidades microbiológicas

El efecto del fuego en la comunidad microbiana es muy diverso, y aunque como el resto de propiedades del suelo, dependerá de la intensidad y la severidad con la que el incendio afecte al suelo, los microrganismos son el componente más sensible del suelo, ya que se ve afectado incluso a temperaturas relativamente bajas (Mataix-Solera et al., 2009). El fuego normalmente reduce la biomasa y diversidad de las comunidades de hongos y bacterias (Barcenas-Moreno et al., 2011a; Neary et al., 1999), que se ven afectadas tanto a corto (efectos del propio fuego), como a largo plazo debido a cambios producidos en el sustrato, microclima y alteraciones en la comunidad vegetal, especialmente en los hongos micorrícicos (HM) (Treseder et al., 2004; fig. 8).

Unos grupos de microorganismos son más sensibles a los cambios producidos por el fuego que otros, como por ejemplo los hongos en comparación con las bacterias y actinomicetos (Guerrero et al., 2005). Por lo que la ratio hongos/bacterias generalmente decrece tras un incendio forestal. De igual manera, la recolonización de las poblaciones de bacterias será más rápida que la de hongos (Barcenas-Moreno y Baath, 2009; Barcenas-Moreno et al., 2011b). Los fuegos de alta intensidad pueden esterilizar parcialmente el suelo, determinando así la capacidad de recolonización después del fuego. En suelos sometidos a temperaturas por encima de 400-500°C se ha observado que los cambios producidos por la temperatura tienen un gran impacto en la recolonización del medio por las poblaciones microbianas (Guerrero et al., 2005). En lo que se refiere a la actividad microbiana (cuantificada como respiración edáfica), esta suele experimentar un incremento inicial que se asocia a la solubilización de compuestos orgánicos por el calor (Barcenas-Moreno et al., 2011a; Guerrero et al., 2005), pero sufre posteriormente un descenso al poco tiempo después del incendio al consumirse esos compuestos lábiles, que puede llegar a mantenerse durante varios meses e incluso años (Mataix-Solera et al., 2009).

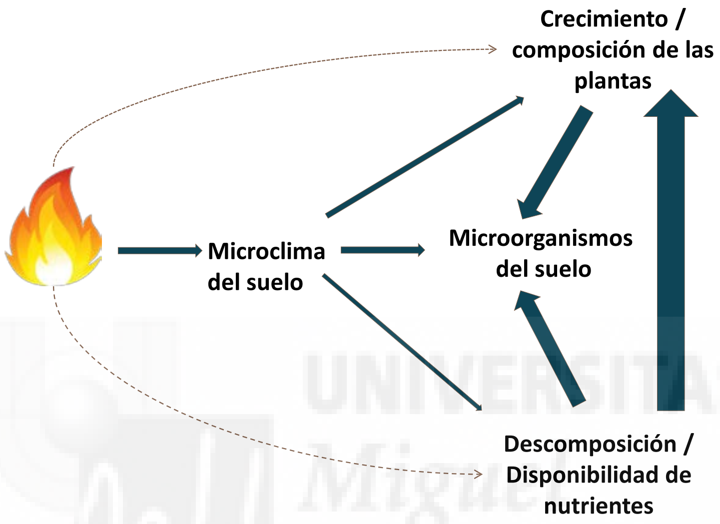


Figura 8: Efectos directos e indirectos del fuego sobre los microorganismos. Las flechas de puntos indican efectos inmediatos, que normalmente son más transitorios. El grosor de las flechas está relacionado con la duración de los cambios producidos tras el incendio. Extraído de Hart et al. (2005).

Las comunidades de organismos autótrofos como cianobacterias y algas generalmente experimentan cambios a corto plazo, recuperándose bastante bien a medio plazo, probablemente por el incremento de la radiación solar que llega al suelo (Acea y Carballas, 1996; Vazquez et al., 1993).

- **Efecto del fuego sobre los hongos micorrícicos**

Los hongos micorrícicos serán sin duda el grupo de microorganismos más afectados por los cambios del fuego producidos en la vegetación, con la que forman asociaciones mutualistas obligatorias para la supervivencia de ambos (hongo y planta). La desaparición total o la parcial destrucción de la planta hospedante condicionará la supervivencia del hongo.

Dependiendo de si las hifas del hongo penetran o no en las células corticales de las raíces de la planta hospedante, hablaremos de endomicorrizas o ectomicorrizas (principalmente Ascomicetos y Basidiomicetos) respectivamente. En cuanto a las endomicorrizas existen diferentes subgrupos basados en las estructuras que desarrollan las hifas diferenciando entre arbusculares, ericoides, arbusoides, monotropiodes y micorrizas de orquídeas (Smith y Smith, 1997), siendo los hongos micorrícicos arbusculares (HMA) los más abundantes. Los ectomicorrícicos (HEM) tienden a ser más abundantes en suelos forestales con horizontes orgánicos bien desarrollados, mientras que los HMA lo son en suelos donde los nutrientes se encuentran en forma mineral, desarrollándose a mayor profundidad (Read, 1991). A priori los HEM se verían más afectados por el fuego, pero sin embargo la intensidad del fuego será el principal factor en determinar la respuesta de los hongos micorrícicos (Smith et al., 2005). Factores como extensión del área quemada o distribución del fuego (manchas de diferentes intensidades, “islas no quemadas” etc.) o el tipo de especie presente en el ecosistema (presencia de rebrotadoras y germinadoras), favorecerán la recolonización de los hongos (Torres y Honrubia, 1997).

Fuegos de baja intensidad en los que sobrevivan las plantas hospedantes no tienen efectos significativos en los hongos micorrícicos y su recuperación se produce a corto y medio plazo (de Roman y de Miguel, 2005), mientras que fuegos de alta intensidad producirán cambios importantes en la comunidad fúngica (Smith et al., 2005). De acuerdo con la ecología de cada tipo de hongo micorrícico, las zonas recientemente afectadas por incendios estarían dominadas por AMF durante las primeras etapas de sucesión y por HEM en etapas más avanzadas (Treseder et al., 2004).

En concreto, los HMA (que son los productores de la glomalina), son relativamente tolerantes a altas temperaturas en el suelo (Pattinson et al., 1999), por lo que los incendios de baja intensidad no causarán daños significativos en la disminución en el número de propágulos (esporas) y en los porcentajes de colonización, impactos observados en incendios de elevada/moderada severidad (Pattison et al., 1999; Treseder et al., 2004). El tiempo que tarden en recuperarse estará directamente relacionado con la severidad del fuego, pero por lo general, la recuperación de los HMA es más rápida que la de los HEM, ya que se verán más favorecidos en las primeras fases tras un incendio debido a la pérdida de la materia orgánica (Treseder et al., 2004).

Glomalina como indicador de salud y recuperación de los suelos tras una perturbación

GRSP es uno de los parámetros del suelo más sensible a las prácticas de manejo agrícola en el suelo (Bedini et al., 2007). La sensibilidad de la GRSP a los cambios en los usos del suelo ha sido probada en muchas ocasiones (Treseder y Turner, 2007). Por ello, son muchos los autores los que proponen GRSP como un indicador de la “salud del suelo”. En un estudio llevado a cabo por Rillig et al. (2003b) en una zona de bosque templado caducifolio y una zona agrícola de Norteamérica, los autores afirman que la relación de la GRSP con la estabilidad de agregados al agua y su respuesta a los diferentes manejos (agrícola, forestal y reforestado), hacen de GRSP un indicador muy útil para evaluar los procesos de regeneración o recuperación de un ecosistema. Los resultados obtenidos por Bonfim et al. (2013) también revelaron que GRSP podría ser usada como indicador de éxito en la revegetación en un estudio llevado a cabo en ecosistemas riparios de bosque atlántico de Brasil. Fokom et al. (2012) utilizaron la GRSP como indicador para evaluar los cambios producidos tras la conversión a suelos agrícolas y su posterior abandono en bosques húmedos de Camerún.

A pesar de la multitud de situaciones en las que podría usarse GRSP como indicador de calidad, hasta la fecha prácticamente la totalidad de los estudios que demuestran la eficacia de GRSP como indicador de la salud del suelo, han sido llevados a cabo en zonas agrícolas. Algunos autores afirman que debido a que los patrones de distribución de HMA, así como los de GRSP están relacionados con la disposición de la ureasa, el C y el N disponible en el suelo, GRSP también podría ser muy útil como indicador de desertificación y degradación del suelo (Singh et al., 2013).

REPELENCIA AL AGUA EN LOS SUELOS

La repelencia al agua o hidrofobicidad es una propiedad de los suelos que reduce la afinidad por el agua, dificultando así la capacidad de humectación de los mismos (fig. 9). Es un fenómeno que ha sido observado prácticamente en todo el mundo, en ecosistemas tanto forestales como agrícolas, en condiciones climáticas y ecológicas muy diversas (Doerr et al., 2000).

Figura 9: Presencia de repelencia al agua en el suelo (SWR; Soil Water Repellency). Fotografía de Victoria Arcenegui Baldó.

Esta propiedad fue descrita por primera vez en 1910 por Schreiner y Shorey (1910), quienes describieron suelos no aptos para la agricultura debido a su resistencia a la humectación, pero fue Jamison (1946) el primero en demostrar que la presencia de repelencia al agua en el suelo reducía la productividad de los cultivos. Esta resistencia de los suelos a la infiltración del agua puede durar desde unos segundos hasta horas, días o incluso semanas y en casos más extremos meses (Dekker y Ritsema, 1994; Doerr y Thomas, 2000). La repelencia al agua suele aparecer en los primeros centímetros del suelo y se caracteriza por ser espacial y temporalmente muy variables (Doerr et al., 2000, Mataix-Solera et al., 2007). En casos de suelos con gran cantidad de compuestos hidrofóbicos puede mostrarse cuando la humedad del suelo desciende hasta alcanzar un umbral crítico y suele desaparecer después de largos periodos de humectación (Dekker et al., 2001). Por lo tanto, el fenómeno no se manifiesta de manera permanente, se presenta con máxima intensidad en las épocas más secas y disminuye, llegando incluso a desaparecer, en las épocas húmedas. Se ha visto que períodos prolongados de sequía generan alta severidad en la hidrofobicidad (Leighton-Boyce et al., 2003), pudiendo ser crítica en algunas regiones áridas donde la mayor parte de las lluvias anuales ocurren en manera torrencial.

Origen y propiedades del suelo influyentes en la repelencia al agua en el suelo

El origen biológico de los compuestos hidrofóbicos es indiscutible. Los compuestos hidrofóbicos naturales son muy abundantes en todos los ecosistemas y normalmente son incorporados al suelo de manera gradual. Estos pueden proceder de diferentes fuentes como exudados de raíces (Dekker y Ritsema, 1996; Doerr et al., 1998)**,** restos vegetales de las plantas que no presentan un grado de alteración muy avanzado, es decir, no corresponden con humus propiamente dicho, también pueden producirse por el metabolismo y/o la descomposición de organismos, principalmente vegetales y microbianos (Doerr et al., 2000), por efecto del calentamiento a determinadas temperaturas (incendios forestales, quemas; DeBano, 2000) o en suelos contaminados con petróleo y/o con sus derivados (fig. 10).

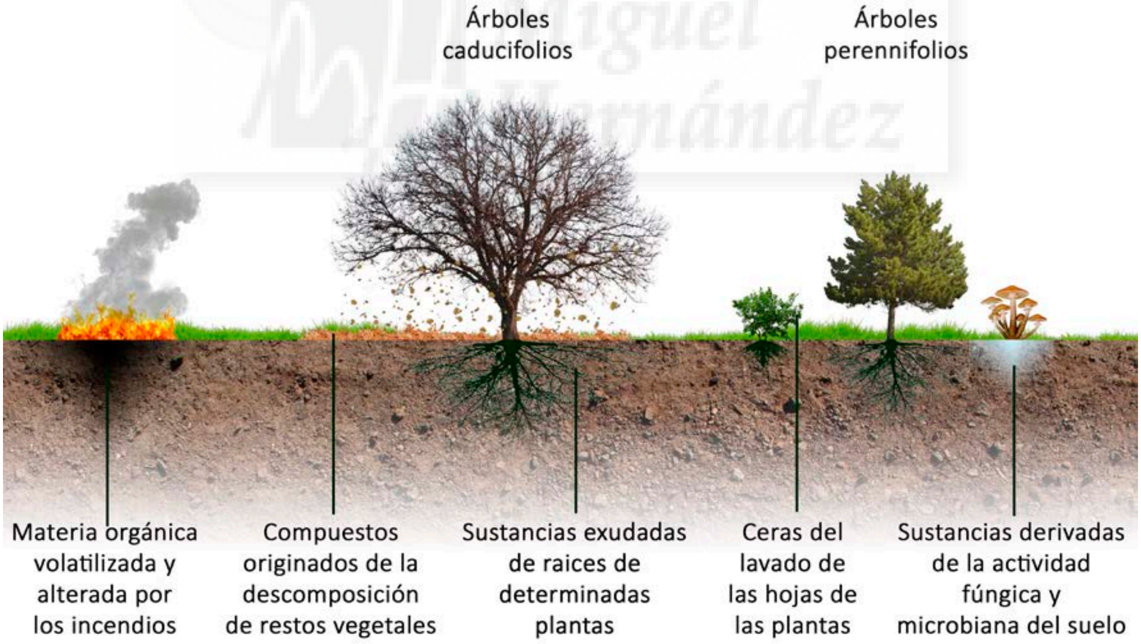


Figura 10: Factores relacionados con la aparición de la repelencia al agua en el suelo. Modificado a partir de Doerr et al. (2000).

El desarrollo de la repelencia depende de las propiedades de la capa externa de las partículas, bastando una capa externa hidrófoba para el desarrollo de la hidrofobicidad. Las plantas hacen el mayor aporte de sustancias que pueden generar repelencia al agua en el suelo.

Prácticamente todos sus órganos contienen compuestos hidrofóbicos que pasan al suelo. La forma en que distintas especies vegetales favorecen o no la hidrofobicidad tiene que ver con la cantidad y el tipo de compuestos orgánicos que se acumulan en el suelo (Mataix-Solera et al., 2007). Se han utilizado varios métodos de extracción para determinar la composición química de los compuestos que pueden estar relacionados con la presencia de hidrofobicidad (Doerr et al., 2005), aunque la composición exacta de todos ellos está lejos de ser establecida completamente. Horne y McIntosh (2000) utilizando diferentes procedimientos de extracción en suelos hidrofóbicos arenosos de Nueva Zelanda, identificaron compuestos implicados en la hidrofobicidad. Los compuestos identificados correspondieron a dos tipos de compuestos: lípidos neutros (constituidos principalmente por alcanos y triglicéridos) y lípidos ácidos, (fundamentalmente cadenas largas de ácidos grasos y una fracción soluble con carácter anfifílico). La presencia y la cantidad de este tipo de compuestos están directamente relacionadas con la especie vegetal, por lo que a menudo la repelencia al agua ha sido asociada a determinadas especies vegetales (Doerr et al., 2000), aunque no puede asumirse que en condiciones naturales siempre vaya a generarse repelencia bajo su influencia. Generalmente la aparición de la repelencia ha sido descrita con mayor frecuencia bajo especies arbóreas, particularmente árboles que producen resinas, ceras o aceites aromáticos. Entre estas especies cabe destacar el pino y el eucalipto (Doerr et al., 1998; Mataix-Solera et al., 2007; Zavala et al., 2014), aunque también ha sido encontrado bajo matorral en diferentes ecosistemas como el mediterráneo (Gimeno-Garcia et al., 2011; Jordan et al., 2008; Verheijen y Cammeraat, 2007) o semidesérticos de chaparral (Brock y Debano, 1990), así como en praderas (Dekker y Ritsema, 1994).

Sin embargo, la aparición de los compuestos en el suelo anteriormente descritos, no siempre implica la aparición de la hidrofobicidad (Horne y McIntosh, 2000), ya que dependerá de la forma y la orientación molecular en la que se encuentren (Doerr et al., 2005), que estará influenciada por las propiedades físicas, químicas y biológicas del suelo.

Entre las propiedades físicas que pueden afectar a la aparición de la hidrofobicidad se encuentran la textura, humedad y temperatura. Generalmente se ha asociado la repelencia a suelos con textura gruesa (DeBano, 2000), factor atribuido a la menor relación superficie/ volumen. Las partículas de arena por ejemplo, requerirán menos cantidad de materia orgánica que las partículas de arcilla para generar una cubierta hidrofóbica, siendo por tanto más susceptibles a su desarrollo. Hecho que ha generado que las arcillas hayan sido utilizadas comúnmente como método para reducir la hidrofobicidad (Blackwell, 2000; Mueller y Deurer, 2011). No obstante, repelencia extrema ha sido encontrada en suelos con altos contenidos de arcillas (Dekker y Ritsema, 1996), hecho que ha sido atribuido en algunos casos a la formación de agregados, que reducirían la relación superficie volumen (Bisdom et al., 1993), y en otros casos, a la gran cantidad de materia orgánica existente en suelo, capaz de recubrir partículas tanto finas como gruesas (Doerr et al., 1996). En estos casos, debido a la mayor superficie de las arcillas, habrá una mayor superficie hidrofóbica en la matriz del suelo que en los suelos de textura gruesa o media, provocando una hidrofobicidad más severa. La mineralogía de la fracción arcilla también influye en la presencia de repelencia al agua (Mataix-Solera et al., 2008)

Anteriormente comentábamos que unas de las características de la SWR es su variabilidad temporal, que está directamente relacionada a la humedad del suelo (Dekker et al., 2001). Este comportamiento se debe fundamentalmente con la interacción de las moléculas orgánicas bipolares con la matriz del suelo. En condiciones de humedad baja, los extremos hidrofóbicos de las moléculas de materia orgánica quedarán libres, interaccionando con la matriz del suelo las partes hidrófilas de las moléculas (Doerr et al., 2000).

Como ya adelantábamos en el apartado de incendios, otro agente que puede generar o modificar el grado de repelencia al agua en el suelo es el fuego (Mataix-Solera y Doerr, 2004). Los efectos del fuego en la repelencia son muy variables. Tras el paso del fuego la repelencia puede aparecer, desaparecer o incrementarse. El principal factor influyente en el comportamiento de la repelencia tras un incendio será la temperatura que se alcance en el suelo (DeBano et al., 1970), aunque su aparición o desaparición tras un incendio es compleja, ya que depende también de las condiciones previas del suelo, el tipo de suelo (Mataix-Solera et al., 2008; 2014), así como la distribución de los fragmentos pequeños de rocas (Garcia-Moreno et al., 2013). Los escenarios son muy variables, pero a nivel general, suele observarse tras el paso del fuego en suelos que previamente eran hidrofílicos (Arcenegui et al., 2008; Debano et al., 1970), o aumentar su persistencia o reducirla en suelos ya hidrofóbicos (Scott, 2000). Estudios de laboratorio han mostrado que la repelencia se intensifica a temperaturas entre los 175-270°C, siendo destruida a temperaturas que oscilan entre los 270-400°C (Arcenegui et al, 2007; Doerr et al., 2000). La temperaturas a las que ocurren esos cambios dependerá principalmente de la duración del calentamiento (Doerr et al., 2004), así como de otros factores, como la presencia de oxígeno. En condiciones insuficientes de oxígeno se ha observado que para la desaparición de la repelencia se necesita alcanzar temperaturas más altas, que pueden rondar entre los 500-600°C (Bryant et al., 2005). Aparte de las condiciones de quemado, factores como: el tipo de suelo (contenido y tipo de arcillas), la cantidad de materia orgánica fresca (hojarasca) acumulada y la acumulación de productos de la descomposición y del metabolismo de los organismos que viven en él, también determinarán el efecto que va a tener el fuego sobre la repelencia al agua del suelo (Arcenegui et al., 2007; Mataix-Solera et al., 2008; 2014).

Entre las propiedades químicas del suelo relacionadas con la hidrofobicidad destaca el pH. Tradicionalmente la repelencia al agua ha sido asociada a suelos ácidos, aunque ya es de sobra aceptado que la repelencia también es común en suelos con pHs básicos (Mataix-Solera et al., 2007). La menor actividad fúngica junto con un menor contenido en materia orgánica típico de suelos de pH neutro o alcalino, podrían ser los factores por los cuales la repelencia es menos común en estos suelos (Hallett y Young, 1999). Uno de los factores que puede explicar el desarrollo de la repelencia en suelos básicos sería la presencia de protones activos y de determinados grupos orgánicos funcionales (Diehl et al., 2010). En suelos ricos en carbonatos se ha demostrado que el desarrollo de la repelencia puede estar asociado a los enlaces producidos entre los iones Ca^{+2} con los extremos hidrofílicos de los lípidos, quedando por tanto libre la parte hidrofóbica en la matriz del suelo (Graber et al., 2009).

Hasta ahora hemos hablado de las plantas como principal factor de la aparición de repelencia al agua en el suelo y de cómo las propiedades del suelo influyen en la misma. Sin embargo, son muchos los autores los que afirman que la relación entre repelencia y las plantas no siempre es directa, su aparición también puede estar relacionada al crecimiento de determinados hongos y microorganismos del suelo, que a su vez pueden estar asociados con diferentes tipos de vegetación (Doerr et al., 2000).

Especies de hongos y microorganismos como *Marasmius oreades* (York y Canaway, 2000), *Penicilliun nigricans* y *Aspergillus sydowi* entre otras han sido descritas por algunos autores como causantes de hidrofobicidad. De hecho, *M. oreades* ha sido considerado como el principal responsable de la aparición de los denominados "anillos de hadas" (York y Canaway, 2000), que son formaciones de anillos concéntricos dentro de los cuales se observa un crecimiento normal de la vegetación (generalmente pasto), seguido por círculo con un crecimiento más exuberante de la misma y rodeado por otro más exterior donde se encuentran los carpóforos del hongo (fig. 11).

Estas formaciones suelen aparecer en suelos arenosos, de baja fertilidad y con poca capacidad de almacenamiento de agua (Jaramillo, 2004).

Figura 11: "Fairy rings" in Saginaw (Michigan, EEUU) by Jeff Schrier, bajo licencia Creative Commons Attribute 3.0 Unported.

Los efectos de los microoganismos del suelo son especie-dependientes, ya que han encontrado tanto especies que la inducen como especies que la reducen (DeBano, 2000). Roper (2004) demostró la función de determinadas actinobacterias en la degradación de compuestos hidrofóbicos.

Novedosos estudios llevados a cabo por Schaumann et al. (2007) y más recientemente por Achtenhagen et al. (2015) han demostrado la contribución de las bacterias a la aparición de la repelencia al agua a través de sus paredes celulares, residuos y respuestas específicas a situaciones de estrés.

Organismos específicos que forman parte de las costras biológicas, en concreto especies de algas, también han sido relacionados con la aparición de la repelencia al agua (Lichner et al., 2013). Pero dado que los microorganismos y los hongos están involucrados también en la descomposición de los compuestos hidrofóbicos (Roper, 2004), es muy difícil determinar que organismos específicos son los responsables de la aparición de la repelencia al agua en el suelo.

Relaciones entre la GRSP y la SWR

La presencia de repelencia al agua en suelo causada por los hongos no sólo ha sido relacionada con su presencia, sino también con su actividad (Doerr et al., 2000). Entre los compuestos procedente de los hongos, hay uno, la glomalina, que es el que más relevancia adquirido en los últimos años. La presencia de glomalina en el suelo ha sido relacionada con la repelencia al agua por diferentes autores. Esto podría deberse a la posibilidad de que la glomalina sea una "hydrophobin": proteínas de tamaño pequeño descubiertas en hongos filamentosos, que debido a sus características (resistencia, adherencia a las superficies, gran superficie activa etc.) podría estar relacionada con la SWR (Rillig, 2005). Sin embargo, los resultados de la influencia de GRSP sobre la SWR son hasta la fecha contradictorios (Feeney et al., 2004; Young et al., 2012).

Técnicas de medida y caracterización de la repelencia al agua en el suelo

Numerosas técnicas basadas en procesos físicos diferentes han sido desarrolladas para la determinación de la SWR, aunque tanto la heterogeneidad química, como la rugosidad macroscópica y microscópica de la superficie del suelo dificultan la aplicación de estos métodos (Tabla 5). Dependiendo de la técnica que usemos obtendremos una información u otra, así distinguiremos entre persistencia, concepto que nos indica el tiempo en el que una gota de agua permanece en la superficie del suelo, y severidad, que nos indica la esfericidad inicial de la gota del agua al entrar en contacto con el suelo, normalmente relacionado con la tensión superficial del suelo (Doerr, 1998; Scott, 2000).

Uno de los métodos más comunes es el test del tiempo de penetración de la gota de agua (WDPT: Water Drop Penetration Time), método muy rápido y sencillo que proporciona información sobre la persistencia de la repelencia. Consiste en poner unas gotas de agua en el suelo y contar el tiempo transcurrido hasta que infiltran en el suelo (Letey et al., 2000). Una particularidad de este método es que puede ser usado fácilmente en campo, algo muy útil cuando se quiere demostrar la presencia de la SWR. En base al tiempo transcurrido la persistencia de la SWR es clasificada en diferentes niveles de repelencia (Bisdom et al., 1993).

El test del etanol (MED: Molarity of an Ethanol Droplet) proporciona información sobre la severidad de la repelencia actual del suelo. Este método usa diferentes concentraciones de etanol para alterar la tensión superficial del líquido, es decir, se determina la molaridad de una solución de etanol a la cual una gota de dicha solución se infiltra de manera instantánea (Doerr, 1998). Una extensión de este concepto es el test de sorptividad realizado mediante un mini-infiltrómeto (Hallett y Young, 1999), donde la sorptividad del agua (influenciada por la repelencia) es comparada a la sorptividad del etanol (no influenciada por la repelencia) para la obtención de un índice de repelencia al agua (R). Otro tipo de medida común es la determinación del ángulo de contacto, tanto directa (CA: contact angle) como indirectamente (CRM: capillary rise method), para el que existen diversos métodos basados en ecuaciones de ascenso capilar, como el método de Letey (1962) o el de Emerson y Bond (1963; Tabla 5).

Tabla 5: Métodos de medida de la repelencia al agua en el suelo y sus principales características.

Método	Información proporcionada	Comentarios generales
Test del tiempo de penetración de la gota de agua (WDPT: Water Drop Penetration Time)	Persistencia	Muy fácil realización y aplicación en campo. Requiere mucho tiempo en suelos muy repelentes
El test del porcentaje de etanol (MED: Molarity of an Ethanol Droplet)	Severidad	Rápido y fácil. Correlacionado con el método del ángulo de contacto.
Test de sorptividad o índice de repelencia R	Severidad de la repelencia a microescala	Dificultad para llevar a cabo el test. Posibles interferencias entre el etanol y el agua en las muestras.
Método del ángulo de contacto (CA: Contact Angle o CRM: Capillary Rise Method)	Severidad	Tiempo de realización. Afectado por la rugosidad de la superficie.

Implicaciones ambientales de la SWR

La hidrofobicidad tiene importantes implicaciones para el suelo y por tanto para el ecosistema. Reduce la infiltración y la disponibilidad de agua para las plantas, incrementa la escorrentía y la susceptibilidad a la erosión, genera grandes pérdidas en la producción agropecuaria y forestal y puede generar vías de flujo preferencial que facilitan el movimiento de agua y de contaminantes a través del mismo (Jaramillo, 2004). Por el contrario, la SWR también puede tener efectos positivos como el aumento de la estabilidad de agregados (Dal Ferro et al., 2012; Mataix-Solera y Doerr, 2004; Vogelmann et al., 2013), las tasas de secuestro de carbono (Müeller et al., 2010) o la reducción de la pérdida de agua del suelo por evaporación (Shokri et al., 2009; Yang et al., 1996), es decir, la presencia de hidrofobicidad en la superficie dificultaría la evaporación de la misma.

Una observación muy común en los suelos propensos a desarrollar SWR, es la aparición de flujos de agua preferenciales debidos a la variabilidad espacial de la intensidad de la repelencia (Caon et al., 2014; Ritsema y Dekker, 1995; Ritsema et al., 1998). Estos flujos consisten en el movimiento vertical del agua a través de una zona hidrofílica rodeada de una matriz hidrofóbica. Dekker y Ritsema (2000) observaron que variaciones espaciales de repelencia a escala de centímetros eran sufiencientes para provocar la aparición de vías de flujo preferencial en el perfil del suelo. Por ello, algunos estudios sugieren que la producción de compuestos hidrofóbicos por algunas especies vegetales, podría ser una estrategia ecológica que les permitiría mejorar la conservación y el almacenamiento del agua por medio de la creación de vías de flujo preferencial que conducirían el agua a más profundidad (secuestro de agua), reduciendo así, la evaporación en el horizonte superior (Robinson et al., 2010; fig. 12). Esta estrategia les proporcionaría una mayor resistencia a la sequía frente a otras especies, pudiendo además, inhibir el crecimiento de raíces superficiales de arbustos y herbáceas bajo su superficie de influencia (Doerr et al., 2000).

Otros autores asemejan las sustancias hidrofóbicas con las alelopáticas, impidiendo el crecimiento y la germinación de plantas competidoras (Stevens y Tang, 1985). Mataix-Solera et al. (2007), sugieren que dicha estrategia competitiva podría tener implicaciones particulares en la sucesión natural de la vegetación después de un incendio, ya que algunas de las especies que presentan mayor repelencia, son frecuentemente usadas en los proyectos de reforestación.

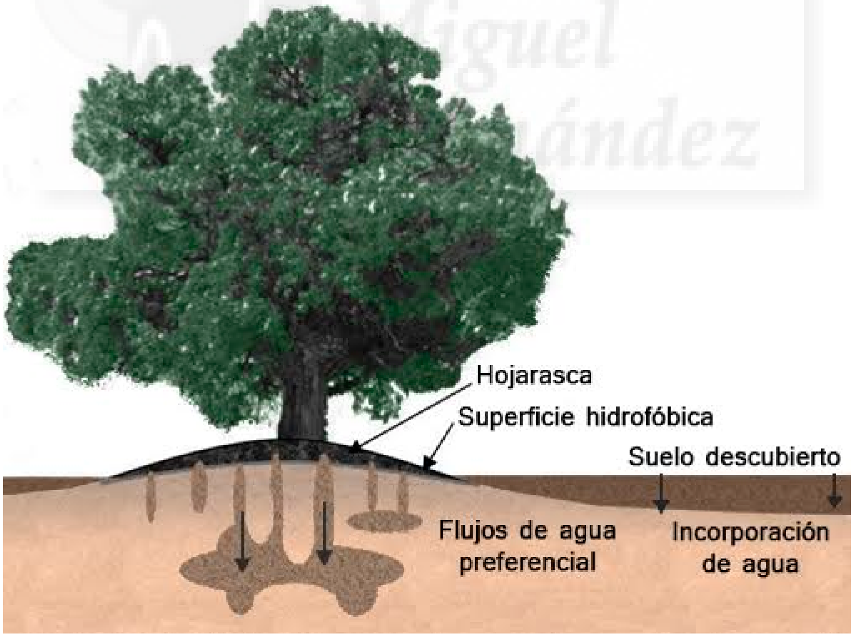
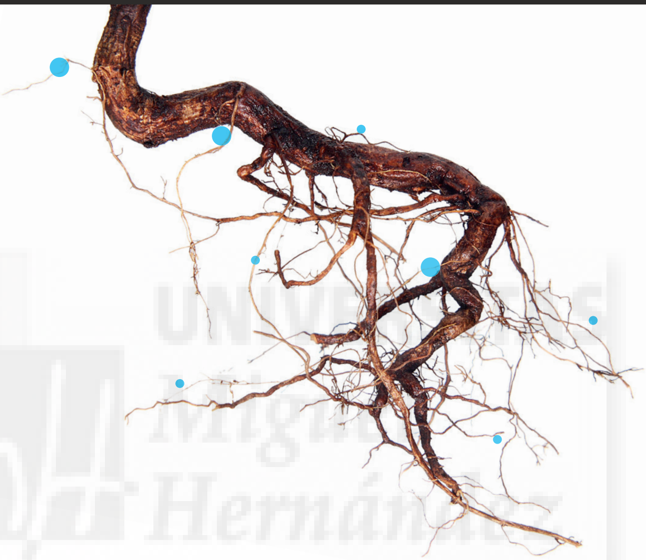


Figura 12: Flujos de agua preferenciales bajo una especia arbórea. La capa de materia orgánica crea zonas repelentes al agua con diferente nivel de severidad. Esto favorece los flujos preferenciales. La infiltración bajo la influencia del árbol fue 3 veces media superior a la del suelo desnudo. Figura extraída de Robinson et al. (2010).

JUSTIFICACIÓN Y OBJETIVOS



En la cuenca mediterránea los cambios en el régimen de incendios y la escasez de agua son dos temas de especial relevancia. En este Año Internacional de los Suelos (2015), la FAO y la Carta Europea de los Suelos nos recuerdan que el suelo es un recurso imprescindible para la vida, siendo a su vez la mayor reserva de carbono orgánico, que es esencial para mitigar el cambio climático y adaptarse a sus efectos, y además fundamental para el almacenamiento de agua y su distribución adecuada.

Las particularidades del clima mediterráneo, caracterizado por una estación estival muy cálida y seca, convierten a la región en una zona de alto riesgo de incendios forestales (Cerdá y Mataix-Solera, 2009). La evaluación de los suelos tras un incendio es imprescindible en la posible toma de decisiones para la rehabilitación de un ecosistema. Por ello, es especialmente importante el desarrollo de indicadores de calidad del suelo que nos proporcionen información acerca del grado de afectación del mismo.

Los estudios sobre severidad del fuego se centran en su mayor parte en el análisis de cambios producidos en parámetros químicos; materia orgánica, disponibilidad de nutrientes, nitrógeno (Couto-Vázquez and González-Prieto, 2006; Fernández et al., 1997; Úbeda et al., 2005), cambios físicos; repelencia al agua en el suelo, estabilidad de agregados (Mataix-Solera y Doerr, 2004; Mataix-Solera et al., 2011) o cambios en la cubierta vegetal (Úbeda et al., 2006). Sin embargo, son de espacial interés aquellos relacionados con la microbiología del suelo, ya que son parámetros muy sensibles a las perturbaciones (Bárcenas-Moreno et al., 2011a, b).

Existen tres categorías principales de indicadores de calidad del suelo: químicas, físicas y biológicas. Los indicadores más interesantes son aquellos que trascienden las tres categorías indicadas, estando por tanto, vinculado a todas sus funciones (FAO). Esta condición la cumple la glomalina del suelo (GRSP), ya que está muy relacionada con propiedades como la materia orgánica del suelo (Rillig et al., 2003), la estabilidad de agregados (Bedini et al., 2009) o la presencia de hongos micorrícicos arbusculares (HMA) (Wright y Upadhyaya, 1996).

Por otro lado, la distribución del agua en el suelo es muy importante para la actividad de los microorganismos y la mineralización del carbono orgánico (Birch, 1958), ya que está condicionada por la calidad de la materia orgánica y su biodisponibilidad para los microorganismos, la temperatura y como decimos, el contenido de agua en el suelo (Kirschbaum, 1995; Leiros et al., 1999; Fang y Moncrieff, 2005). Como ya apuntábamos, el suelo es el encargado del almacenamiento y la distribución del agua. En este sentido, llama la atención que en un clima semiárido como el nuestro, los suelos en determinadas ocasiones y bajo determinadas condiciones manifiesten una falta de afinidad por el agua, propiedad conocida como repelencia al agua en el suelo o hidrofobicidad**.** La repelencia al agua en los suelos es un fenómeno frecuente (Doerr, 2000), del cual se conoce y entiende poco en ambientes naturales, que puede tener efectos notables sobre la productividad y la producción agropecuaria y forestal, sobre la erosión y sobre el comportamiento hidrológico del suelo (DeBano, 2000; Doerr, 2000). Su variabilidad, la manera como se expresa en el campo y el comportamiento que presenta este fenómeno hace difícil su detección, su evaluación y su manejo. En zonas semiáridas, donde el factor limitante para el crecimiento y desarrollo de la vegetación es el agua, esta propiedad juega un papel fundamental y existen muchos aspectos sobre su presencia y causas todavía desconocidas.

Para poder comprender mejor las implicaciones reales de esta propiedad en la producción vegetal y en la sucesión de la vegetación tras el paso del fuego en el monte, es necesario conocer su papel e implicaciones en ambientes naturales y particularmente en ambientes semiáridos.

Una de las consecuencias de la repelencia en el suelo no estudiadas hasta la fecha, es el posible impacto sobre la estructura de las comunidades de microorganismos, ya que son un factor del suelo sensible a la variación de las condiciones físicas (como la humedad) y químicas (como el pH y la materia orgánica) del suelo.

En este sentido en esta tesis se ha estudiado por un lado el potencial de la GRSP como posible indicador de calidad y salud de los suelos tras un incendio forestal, y por otro, los factores implicados en el desarrollo de la repelencia al agua en el suelo (entre los que se encuentra la GRSP), así como las implicaciones ecológicas para la microbiología del suelo, elemento fundamental de los suelos.

Para contribuir al conocimiento de los aspectos mencionados, los objetivos específicos de esta Tesis Doctoral son los siguientes:

1) Analizar los cambios inducidos por las temperaturas alcanzadas en el suelo en el contenido de glomalina en diferentes tipos de suelos.
2) Determinar los efectos inmediatos de los incendios forestales en el contenido de glomalina del suelo bajo la influencia de dos coberturas vegetales diferentes (pinar frente a matorral).
3) Conocer los efectos a medio plazo de un incendio forestal sobre el contenido de glomalina del suelo bajo la influencia de dos tipos de coberturas vegetales (pinar frente a matorral).

4) Estudiar la relación entre la glomalina y la repelencia al agua en el suelo y contribuir al conocimiento de los principales factores biológicos y químicos implicados en su desarrollo y las relaciones entre dichos factores en condiciones naturales.
5) Conocer los efectos de la repelencia al agua en el suelo sobre las comunidades microbiológicas del suelo y viceversa; entender mejor la implicación de los microorganismos del suelo en el desarrollo de la repelencia al agua en el suelo.

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(http://www.fao.org/soils-portal/degradacion-del-suelo/evaluacion-de-los-indicadores-globales-de-la-salud-del-suelo/es/)

CAPÍTULO 1

Glomalin-related soil protein response to heating temperature: a laboratory approach

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ABSTRACT

Forest fires are a recurrent natural phenomenon in the Mediterranean basin. Fires can affect physical, chemical and biological soil properties. The effects on soil properties are closely controlled by fire severity, which is a consequence of temperatures reached and the length of residence of heat in the soil. In this study, the response of Glomalin-Related Soil Protein (GRSP) -a glycoprotein produced by arbuscular mycorrhizal fungi- to heating temperature has been studied. Laboratory heating treatments were carried out at 180, 200, 250, 300, 400 and 500°C in soil samples from eight different sites in SE Spain. The sites selected had mainly different soil characteristics. The results of heating on Soil Water Repellency (SWR) and Soil Organic Carbon (SOC) were also included in the study. GRSP response to temperature was different between sites. Redundancy Analyses divided sandy soils from the rest of soil types. Total Content of Aggregates (TCA), SOC, clay and sand content were the most significant properties explaining the response of GRSP to heating treatments. Results showed that GRSP was affected even at low temperature. SOC response to temperature was very similar between sites. SWR did not appear after heating in wettable soils and disappeared in water repellent ones at temperatures over 200°C. Our results indicate that GRSP could provide relevant information about fire severity.

Key words: arbuscular mycorrhizal fungi, glomalin-related soil protein, soil water repellency, soil aggregates, fire severity.

INTRODUCTION

It is widely accepted that studying soil requires an interdisciplinary view and soil should not be understood like a single science (Brevik et al., 2015), especially after perturbations like fires. Changes in soil properties after a fire have direct and indirect consequences on water resouses (Keestra et al., 2014), vegetation and microorganisms (Guénon and Gross, 2015; Guénon et al., 2013; Wang et al., 2015) between others. Many of the soil services, which are essential to humans and environment, are provided by soil biota. In this sense, soil microorganisms and the soil properties related (like GRSP) can be very useful to identify the soil quality (Rillig et al., 2003, Morugán-Coronado et al., 2013; Bochet 2015; Zornoza et al., 2015).

Glomalin is a glycoprotein produced primarily by arbuscular mycorrhizal fungi (AMF) (Wright and Upadhyaya, 1996). It is iron-containing and is found within AMF hyphal and spores walls (Wright and Upadhyaya, 1996; Driver et al., 2005). Residues of glomalin are deposited within the soil when hyphae die and decompose (Treseder and Turner, 2007). Glomalin quantified and operationally detected in soils is called glomalin-related soil protein (GRSP) (Rillig, 2004). It is quite recalcitrant and decomposes slowly in laboratory incubations (Steinberg and Rillig, 2003). Most proteins are denatured in a range between 50- 80°C (Keeley, 2009). However, GRSP is extracted from soil at 121°C (Wright and Upadhyaya, 1996). So, it can be said that GRSP is heat stable and has a relatively long soil life (Steinberg and Rillig, 2003).

Arbuscular mycorrhizal fungi (AMF) form mutualistic associations with about 70% of plant families (Newman and Reddell, 1987) and are abundant in all major terrestrial biomes (Treseder and Cross, 2006), therefore, this glycoprotein is present in almost all the terrestrial ecosystems.

It is being widely studied due to its implication in carbon and nitrogen storage (Treseder and Turner, 2007), and also for its role in aggregate stability (AS) (Rillig, 2004), Soil Water Repellency (SWR) (Lozano et al., 2013) and sequestration of potentially toxic elements in soils (Cornejo et al., 2008).

Many soil properties are directly affected by fire (Certini 2005). These changes are directly and indirectly connected. Understanding what is happening to soil properties after a wildfire is crucial in taking appropriate decisions about possible post-fire treatments. However, studying wildfire effects on soil properties is not an easy issue. Complex relationships between variables and the diversity of natural systems make results difficult to analyse and it is often difficult to make comparisons between studies. Burning experiments in laboratory are needed to study the direct effects of temperature on soil properties. Laboratory experiments also provide relevant information that can be useful in making soil samplings more accurate (Badía et al., 2014). Thus, many authors have studied the effect of temperatures on soils properties. There are laboratory studies about how temperatures influence different soil properties such as soil organic matter, aggregate stability (AS), soil water repellency (SWR) (Mataix-Solera et al., 2008; Aznar et al., 2014; Badía et al., 2014; Certini, 2005). Nonetheless, to the authors' knowledge, no laboratory studies have been conducted to date on the effect of heating on GRSP.

The main objective of this research was to examine the temperature-induced changes on GRSP content on different soils. To achieve this, soil samples from 8 different sites were burnt under laboratory conditions at different temperatures (180-500°C). SWR and SOC (common properties associated with GRSP) response to temperature were also studied. Finally, advantages and disadvantages of the study of GRSP, SWR and SOC immediately after a fire under field conditions are discussed.

MATERIAL AND METHODS

Study sites and soil sampling

Soil samples were collected from 8 forest sites in Alicante province (SE Spain) to cover a representative spectrum of the main soil types of the region (Table 1 and fig. 1). Samplings were carried out between February and March 2013. The main characteristics of soil samples are described in Table 2. At each site, samples were taken from the first 2.5 cm of the A mineral horizon by pooling subsamples from different places to have finally 3 composite samples per site. Soil samples were dried at room temperature (20-25°C) to a constant weight and sieved (< 2 mm) to eliminate rock fragments before soil analysis.

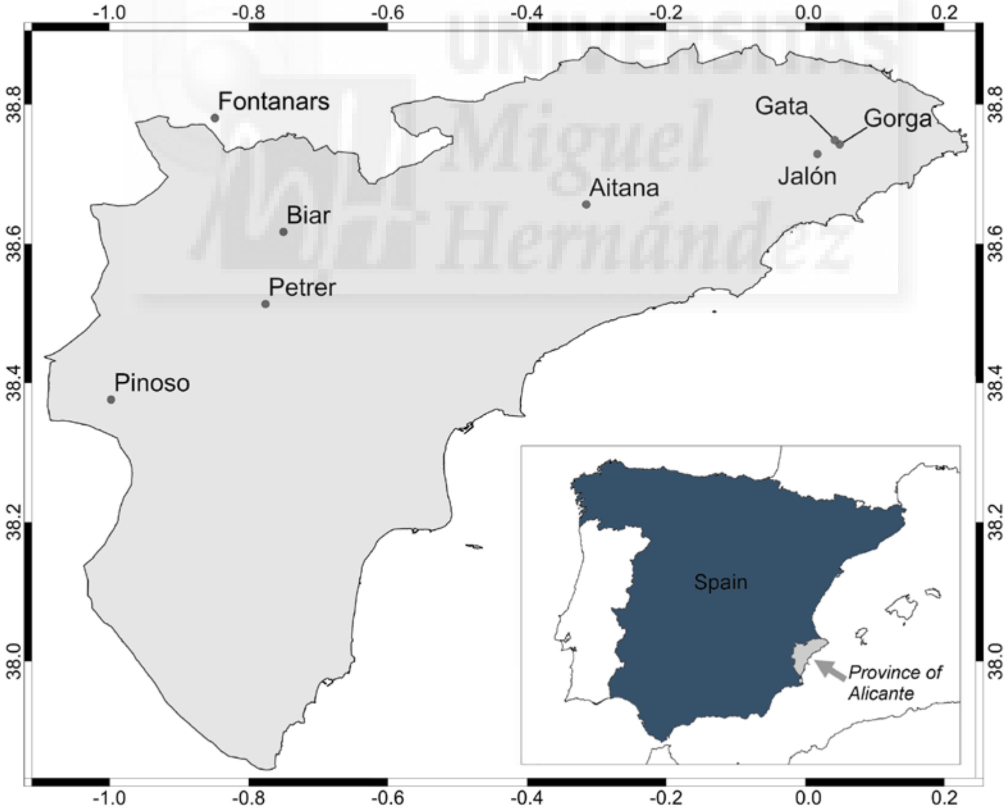

Figure 1: Study area and location of sampling sites.

Table 1: Main characteristic of the sampling sites

Site	Location Coordinates	Annual rainfall	Dominant species	Lithology	Soil type*
Petrer	38°30'48''N, 0°46'33''W	371	*Pinus halepensis, M. Rosmarinus officinalis, L*	Cretaceous limestone	Psamment
Fontanars	38°46'50''N, 0°50'55''W	454	*P. halepensis, Quercus coccifera L, R. officinalis*	Cretaceous limestone	Xerorthent
Biar	38°37'1''N, 0°44'60''W	486	*P. halepensis, R. officinalis*	Tertiary limestone	Haploxeroll
Gorga	38° 43’94"N; 0° 22’58’’E	500	*P. halepensis R. officinalis*	Jurassic limestone	Xerorthent
Jalón	38°43'44''N, 0°01'3''E	656	*Q. coccifera P. halepensis,*	Cretaceous sandstone	Rhodoxeralf
Pinoso	38°22'34''N, 0°59'52''W	260	*P. halepensis, R. officinalis*	Tertiary limestone	Xerorthent
Aitana	38°39'23''N, 0°18'53''W	706	*Q. ilex L, P. halepensis, R. officinalis*	Cretaceous limestone	Calcixeroll
Gata	38° 44' 56"N; 0° 2' 32"E	692	*P. halepensis,Q. coccifera R. officinalis*	Cretaceous limestone	Rhodoxeralf

*SSS: Soil Survey Staff (2014), *P: Pinus, Q: Quercus, R: Rosmarinus*

Heating treatments

Around 10 g of each soil sample were placed in a porcelain container (8.5 cm diameter, 3.5 cm depth). Heating treatments were carried out in a pre-heated muffle furnace (Nabertherm, P320, Bremen, Germany) at 180, 200, 250, 300, 400 and 500°C during 20 minutes. Three replicates per sample and heating temperature treatment were made.

Analytical methods

Characterization of soils was carried out on air-dried samples (Table 2), which included pH (1:2.5 w/v, distilled water), texture determined by Bouyoucos method (Gee and Bauder, 1986), soil organic carbon (SOC) by potassium dichromate oxidation (Nelson and Sommers, 1982) and carbonates by method described in Hulseman (1996).

GRSP extracted in this study was Easily Extractable Glomalin (EEG), which corresponds with the last fraction deposited into the soil and is extracted in just one extraction cycle. EEG was extracted from 0.25 g subsamples with 2 ml citric acid buffer (20 mM), pH 7.0 at 121°C for 30 min in autoclave. Janos et al. (2008) observed, in a soil with high content of soil organic matter, a progressive decrease in GRSP with delayed centrifugation after autoclaving. So, after extractions, samples were immediately centrifuged at 3000 rpm (during 15 minutes) to avoid this problem. Then, the supernatants were isolated from the soil. Protein in the supernatant was determined by Bradford assay (Wright and Upadhyaya, 1996). Bovine serum albumin, a protein of similar molecular weight to glomalin (Rosier et al., 2006), was used as standard.

The persistence of SWR was measured by the Water Drop Penetration Time (WDPT) test (Wessel, 1988). This involved placing 3 drops of distilled water (~0.05 ml) onto the sample surface and recording the times required for their complete penetration. The average time for triplicate drops has been taken as the WDPT value of a sample, with WDPT ≤ 5 s representing wettable and WDPT >5 s water repellent conditions. The logarithm of the WDPT value in seconds has been used; being water repellent if the value of log (WDPT) is >0.7 (Bisdom et al. 1993).

Soil aggregate stability (AS) and total content of aggregates (TCA) were studied in the macroaggregate fraction of 4-0.25 mm and determined with the rainfall simulator method according to Roldán et al. (1994) and based on the method of Benito et al. (1986). This method examines the proportion of macroaggregates that remained intact after an artificial rainfall. Four grams of soil material (4-0.25 mm) were exposed to an energy of 270 J m^{-2} applied by artificial rainfall. The material remaining within the sieve (remaining aggregates, mineral particles and organic debris) was dried and weighed. Then, this material was washed until only mineral particles and organic debris remained. The dry weight of these materials was subtracted from the previous one to calculate the aggregate weight (%) within a sample before and after artificial rainfall (AS). The method also allows for calculating the proportions of sample that is forming aggregates (TCA) by differences of weights. Results are based on the mean value of three replicate experiments per soil sample.

Statistical analysis

To evaluate the response of GRSP and SOC of the different soils at each heating treatment, GRSP contents and SOC were converted to relative contents. Content of SOC and GRSP for each treatment were divided by the initial content of the sample before the treatment (control samples). Redundancy analysis (RDA) was used to seek the combinations of explanatory variables (soil initial characteristics) that best explain the variation of the dependent matrix explore (GRSP content at different temperatures). Samples with similar trends in GRSP content at different temperatures have similar scores and will therefore be grouped closer together when plotted. Soil properties were tested for significant contributions to the variation in the GRSP data using the Monte Carlo permutation test ($P < 0.05$). Soil properties are represented by vectors distributed on 2 axes. The correlation with the axis is measured by both the angle with the axis and magnitude of the vectors. Small angle and great magnitude means a greater correlation. RDA was performed using CANOCO for Windows, Version 4.5.

RESULTS

Soil characteristics

To study the response of GRSP content to heating treatments, different types of soils were used in this study, covering at the same time, a range of the most common soils in the Province of Alicante, including 3 Xerorthents, 1 Xeropsamment, 1 Haploxeroll, 1 Calcixeroll and 2 Rhodoxeralfs (Table 1). Four of them have a more sandy texture (Petrer, Fontanars, Biar and Gorga). Gata has a clay texture and the other sites have clay loam texture (Jalón, Pinoso and Aitana; Table 2). All soils are calcareous with pH > 7 and the SOC varies from low (1.58% in Petrer) to high (9.76% in Aitana). A high variability was also found in initial GRSP content, which vary between 1067 µg/g (Gorga) to 2744 µg/g (Aitana). The lowest TCA and AS generally corresponded to sandy soils (Table 2). SWR was present in just three of the soils and it seems to be related with the SOC (Aitana and Gorga) or soil texture (Petrer). Carbonate content varies greatly between them -despite all being developed over calcareous parent material- mainly due to the soil age and microclimatic conditions (Table 2).

Table 2: Main characteristic of the soil samples used (0-2.5 cm depth from the mineral soil horizon). Mean values ± standard deviation (n=3) of each site.

Site	AS (%)	TCA (%)	SOC (%)	GRSP (µg/g)	pH	Carbonates (%)	WDPT (s)	Texture* (sand, silt,clay)
Petrer	61.6 ± 2.6	60.6 ± 4.1	1.6 ± 0.3	1346 ± 9	8.3 ± 0.2	32 ±4	15 ± 5	Sand (90, 7, 3)
Fontanars	45.6 ± 4.2	56.6 ± 6.7	2.9 ± 0.2	1745 ±7	8.2 ± 0.2	64 ± 5	1 ± 0	Sandy loam (72, 21, 7)
Biar	45.9 ± 15.5	58.3 ± 5.9	2.5 ± 0.8	1806 ± 26	8.2 ± 0.1	43 ± 4	1 ± 0	Sandy loam (70, 23, 7)
Gorga	84.1 ± 1.3	76.3 ± 0.01	3.8 ± 0.1	1067 ±23	7.9 ± 0.1	57 ± 5	1 ± 0	Sandy silty loam (50, 41, 9)
Jalón	55.2 ± 9.7	95.6 ± 0.68	2.2 ± 0.6	1613 ±52	7.8 ± 0.2	2 ± 1	1 ± 0	Clay loam (43, 33, 24)
Pinoso	70.6 ± 7.2	80.6 ± 5.9	3.7 ± 1.2	1697 ±50	8.1 ± 0.1	40 ± 5	3 ± 1.9	Clay loam (40, 36, 24)
Aitana	82.3 ± 1.9	82.9 ± 4.4	9.8 ± 0.2	2744 ±87	7.7 ± 0.1	6 ± 1	175 ± 43	Clay loam (44, 31, 25)
Gata	91.0 ± 0.3	80.8 ± 0.1	7.9 ± 0.3	2209 ±76	7.8 ± 0	47 ± 4	9 ± 2	Clay (19, 38, 44)

*: Sand (2-0.05), Silt (0.05-0.002), Clay (< 0.002). AS: Aggregate Stability, TCA: Total Content of Aggregates, SOC: Soil Organic Carbon Content, GRSP: Glomalin-Related Soil Protein, WDPT: Water Drop Penetration Time (seconds)

Laboratory heating treatments

- *GRSP curve trends descriptions*

GRSP concentrations were sensitive to temperature of heating. The GRSP response was different between soil types. Thus, curve trends were placed in two different graphs to appreciate better the results. The figure 2a represented the sandy soils, while in 1b the clay loam and clay soils were represented. Changes in the concentrations were observed even at 180°C in some soils. A peak of GRSP content was found at 200°C in sandy soils (fig 2a). In general, the GRSP concentrations decreased at 250°C, although such decreases also varied between soils. The highest decrease at that temperature was observed in Aitana soil (65% of concentration disappeared), in contrast to Petrer and Gata, where the concentrations continued being higher or similar to the initial ones respectively. At 300°C, a different response between clay loam and clay versus sandy soils was observed. In clay loam and clay soils, the decrease of GRSP content was much higher (> 55%; fig. 2b) than in sandy soils (< 50%, fig. 2a). GRSP was very low at 400°C (more than 95% of the concentration disappeared) and practically vanished at 500°C (>97%) in all studied soils (fig. 2a and b).

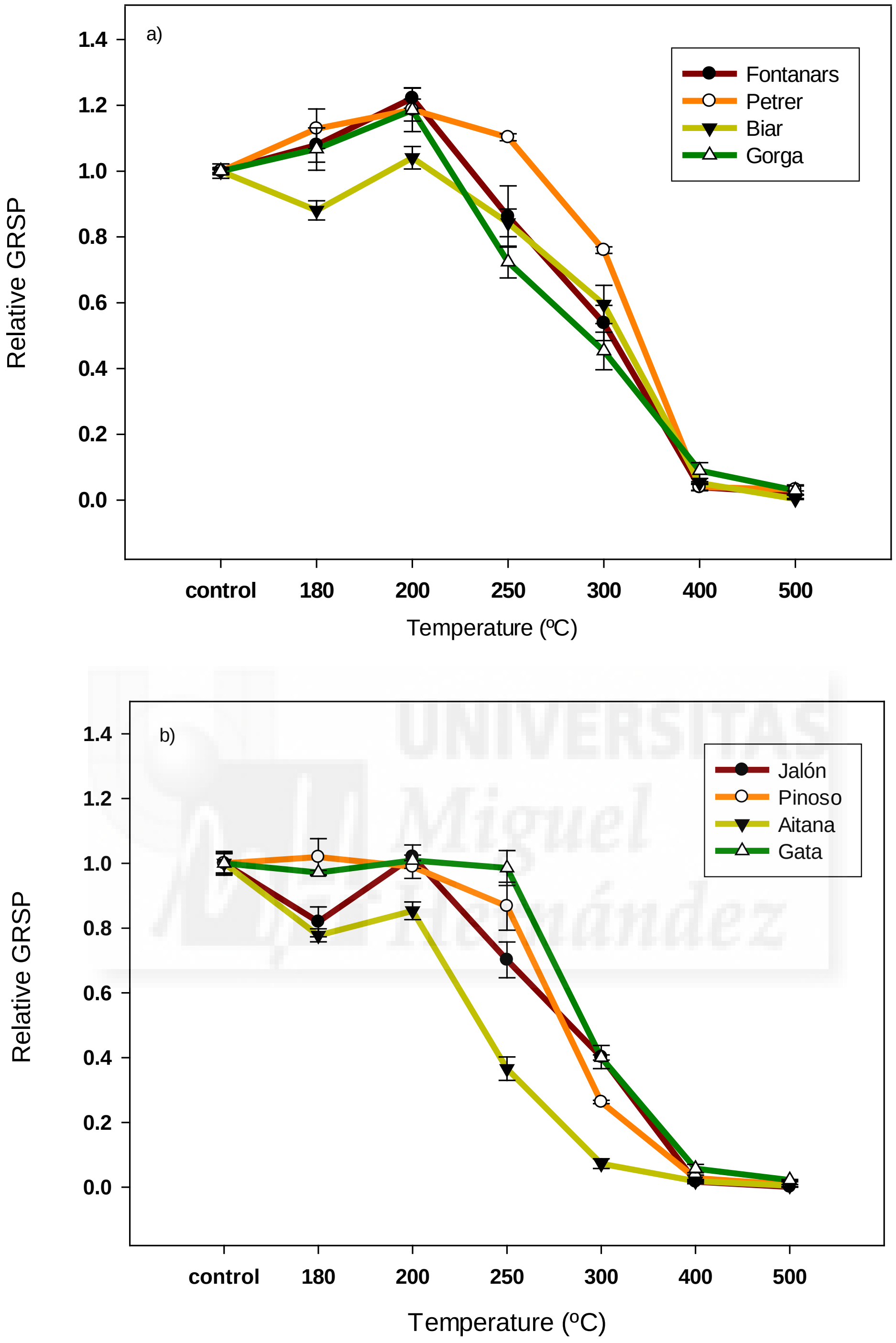

Figure 2: (a) GRSP relative concentrations (± standard deviation) vs temperature. Petrer, Fontanars, Biar and Gorga sites. (b) GRSP relative concentrations (± standard deviation) vs temperature Jalón, Pinoso, Aitana and Gata sites.

- *Multivariate analysis*

The RDA performed with the GRSP relative concentrations at each temperature showed that the first two axes explained 81.4% of the total variation, where soil samples were clearly clustered by soil texture. Axis 1 separated sandy soils samples from clay loam soil samples and explained 74.1% of the variation, whilst Axis 2 explained 7.3% of the variation (fig. 3). Eight different parameters were introduced to try to explain the variability of the results (fig. 3). Except for pH and AS, all the soil parameters were significant ($P < 0.05$) in explaining the results. TCA was the variable which had the greatest influence in explaining the variation in the GRSP concentrations. TCA, SOC and GRSP showed a positive association with axis 1, while sand and pH showed a negative association. Clay showed a positive correlation with Axis 2 (fig. 3).

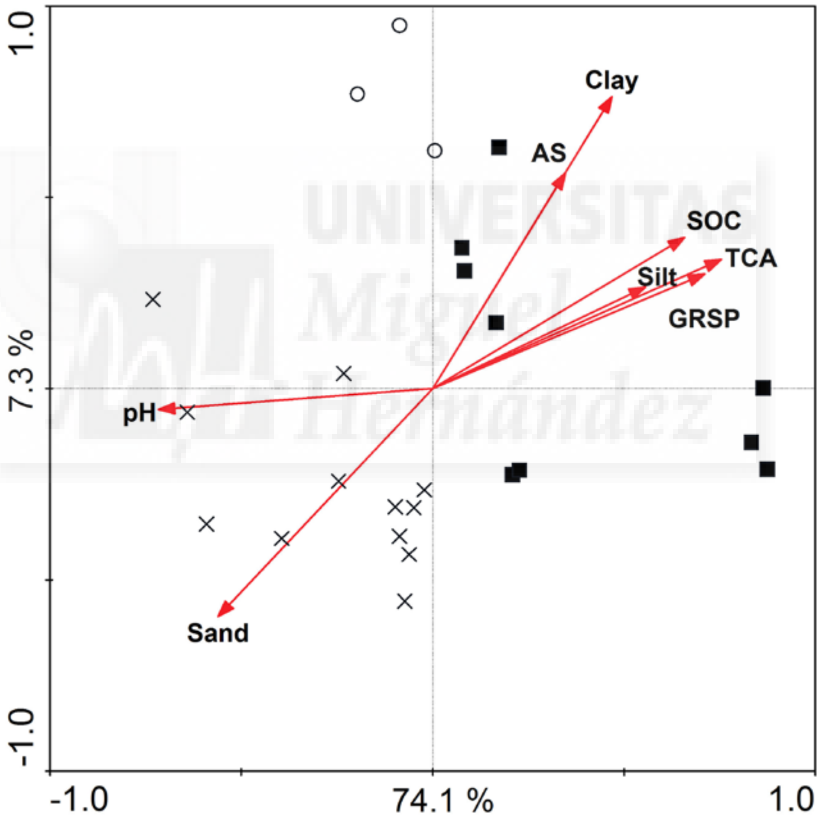

Figure 3: Samples and soil parameters biplots from RDA performed on the relative concentrations of GRSP at different temperatures and sites. Soil samples organized by soil texture: X-mark: sandy samples, squares: clay loam samples, empty circles: clay samples. SOC: soil organic carbon, TCA: total content of aggregate, AS: aggregate stability, TCA: total content of aggregate, GRSP: glomalin-related soil protein.

- *SWR and SOC response to temperature of heating treatments*

In contrast to the GRSP curves, the relative variation of SOC followed a very similar trend between samples (fig. 4). SOC contents were quite constant below 200°C (except Biar and Gata). At 250°C, 30-40% of SOC content was lost and it practically disappeared at 400°C.

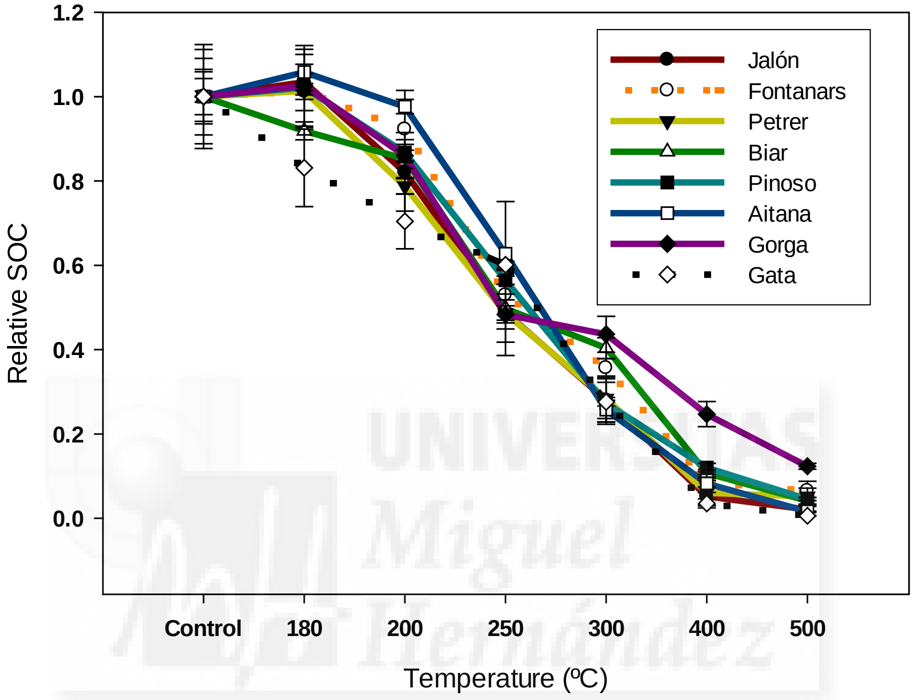

Figure 4: SOC (soil organic carbon) relative content (± standard deviation) vs temperature.

SWR did not appear in original wettable samples after heating but it was present in the water repellent ones until 200°C (Table 3), over this temperature, water repellent samples became wettable.

Table 3: Pre- and post-heating values of WDPT (water drop penetration time) test in seconds for all sites and treatments. Mean values ± standard deviation of three tests on each of three subsamples.

			Temperature				
Site	**Control**	**180°C**	**200°C**	**250°C**	**300°C**	**400°C**	**500°C**
Petrer	14.7 ± 5.5	6.7 ± 0.6	4.6 ± 2.7	<1	<1	<1	<1
Fontanars	<1	<1	<1	<1	<1	<1	<1
Biar	<1	<1	<1	<1	<1	<1	<1
Gorga	<1	<1	<1	<1	<1	<1	<1
Jalón	<1	<1	<1	<1	<1	<1	<1
Pinoso	<1	<1	<1	<1	<1	<1	<1
Aitana	175.7 ± 43.1	301.7 ± 150.5	320.3 ± 93.3	<1	<1	<1	<1
Gata	9 ± 2.6	24.2 ± 4.3	20.4 ± 8.5	<1	<1	<1	<1

DISCUSSION

GRSP response to heating treatments

Many works and reviews about the effects of fire on soil properties under laboratory conditions are available in the literature (González-Pérez et al., 2004; Certini, 2005; Terefe et al., 2008; Mataix-Solera et al., 2011). However, laboratory studies about how GRSP content is affected by temperature of heating have not been reported to date.

GRSP content was clearly affected by heating and its response differed between soil types. The GRSP curve trends appear to be closely related to the initial soil properties and characteristics (fig. 2 and 3). Soils samples were clearly grouped by soil texture (fig. 3). Soil texture influences the GRSP response to temperature and extraction. Janos et al. (2008), in a work in which the aspects of glomalin extraction and measurement were examined, suggested that sandy soils are the easiest from which to extract GRSP. This would explain why an increase of GRPS concentration at low temperature (180°C) appeared in soils that have a low percentage of clay and high content of sand (Petrer, Fontanars and Gorga). The clay influence can be enhanced in the presence of Ca^{+2} and Mg^{+2}, which would serve as a metal bridge between glycoproteins and clay minerals (Halvorson and González, 2006).

According to the RDA results, TCA was the variable with the most influence on the variations of GRSP content to heating. Aggregates formation and stabilization depend on many soil characteristics, the most important being soil organic matter and soil texture (Amezketa, 1999), even GRSP is also commonly related with AS (Singh et al., 2013). Aggregation would explain the GRSP curve trends below 250°C. GRSP involved in the aggregates could be more protected than the rest of GRSP present in soil. Part of this GRSP could also correspond to an older GRSP, which is more difficult to extract. This type of GRSP is not measurable with the EE-GRSP protocol; longer and repetitive cycles for the total extraction are needed (Wright and Udpdhayaya, 1996). GRSP is heat stable and temperature resistant (Steinberg and Rillig, 2003). Thus, this older more recalcitrant GRSP would become measurable (at highest temperatures) with the breakdown of aggregates when the organic matter combustion takes place, which could be the explanation for the increases observed in most cases in the temperature range between 180 and 200°C (fig. 2). Many authors (Badía and Martí, 2003; García-Corona et al., 2004; Marcos et al., 2007) have described a decrease in AS with temperature in laboratory heating. Zavala et al. (2010) did not find a decrease in AS at temperatures below 150°C, but did at 250°C in sandy loam and loamy sandy soils. Changes to the particle size distribution towards the sand fraction and in consequence in aggregates could be also involved. Giovannini et al. (1988), described changes in particle size distribution beyond 220°C in sandy loam and silty clay soils. Changes would make the GRSP extraction easier.

The highest rate of decrease in GRSP was observed in Aitana soils (fig. 2a). Aitana was also the soil with the highest SOC content (9.8%; Table 2). At the same heating temperature of exposition, soils with higher quantities of SOC could reach higher burn severity than soils with a lower SOC content. Therefore, SOC could be increasing the rate of decrease of GRSP concentration with temperature.

SWR and SOC response to heating

Many researchers (see reviews of Certini, 2005, González-Pérez et al., 2004) have studied the effect of heating on SOC. In laboratory conditions, consumption of organic matter begins in the 200-250°C range being completed at around 460°C (Giovannini et al., 1988), which is in accordance with our results. SOC curves followed a very similar trend and relevant effects at low temperatures were not observed. Neither qualitative nor quantitative changes in SOC have been observed at low temperature ranges between 150 and 220°C (Fernández et al., 1997, Badía and Martí, 2003). Main changes occur at intermediate temperatures (Certini, 2005). SOC decreased between 30-40% at 250°C. Fernández et al. (1997) found similar results, they observed that 37% of SOC was lost at 220°C.

It is known that SWR is present in many forest soils and that it can be increased or eliminated after a fire (DeBano 2000; Doerr et al., 2000). It is considered that SWR increases at low temperatures (175- 200°C) and disappears when 270- 300°C are exceeded (DeBano, 1981;

Zavala et al., 2010). However, the relationship between SWR response and temperature is not as easy as appears. SWR development, induction, enhancement or decrease, depends on many other factors such as the nature of atmospheric heating (Bryant et al., 2005), the quantity and quality of soil organic matter content, soil moisture (Doerr et al., 2000), soil texture (Arcenegui et al., 2007), mineralogy of clay fraction (Mataix-Solera et al., 2008) or even rock fragments (Gordillo-Rivero et al., 2014). In our case, SWR increased at low temperatures when natural SWR was initially present and was mainly caused by the high SOC content (Gata and Aitana samples, clay and clay loam soils respectively). On the contrary, a decrease was observed when the main factor affecting natural SWR was the texture (Petrer samples, sandy soil). Doerr et al. (2004) also observed an increase at low temperatures in SWR in natural water repellent soils when burnt at different temperatures during 20 minutes. However, fire does not always induce changes in SWR. In this study, no heating effects have been observed in original wettable samples (Table 3). Busse et al. (2005) also did not find any effect of heating in wettable soil samples with clay loamy texture.

Advantages and disadvantages of the study of GRSP, SWR and SOC immediately after a wildfire under field conditions

The study of some soil parameters like SWR, SOC and GRSP content after a wildfire might supply valuable information about temperatures reached in soil during combustion. However, under field conditions, there are many factors involved that often make it difficult to put into practice.

Soil organic matter is a commonly used indicator in explaining fire severity in soils (Turner et al., 1994; Ryan, 2002; Keeley, 2009). Developing sensitive indicators at low temperatures is needed, as the most typical soil surface temperatures for forest fires do not pass 300°C (Gimeno-García et al., 2004), although, much higher temperatures have been recorded in some cases (Dunn and DeBano, 1977). Nevertheless, as it has already been discussed, significant changes in SOC appear at intermediate temperatures. In general, soil properties such as texture seem not to influence the SOC response to temperature, which is an advantage. The main problem of SOC as an indicator of fire severity may reside in the external inputs from the forest necromass and semipirolized ash (Fernández et al., 1997, Jordán et al., 2011), which can alter the results. In fact, increases in SOC after a wildfire have been described (Mataix-Solera et al., 2002). Thus, conditions in the field frequently do not coincide with the laboratory conditions what makes it difficult to determine the real fire severity in soil.

SWR is also commonly used as a describing factor in the fire severity indexes (for example: USDA, Post fire index (PFI) by Jain et al., 2012) since SWR is a property particularly affected by fire (Soto et al., 1991). In fact, Doerr et al. (2004) proposed that post-fire SWR could be used as simple indicator of soil temperatures reached during a fire but only within repellency prone environments. Its limitations reside in the fact that its appearance after a wildfire is not always predictable. Fire does not always induce changes in SWR, which supposes a great limitation in the use of SWR as a severity indicator. SWR measurements are really easy to carry out in both field and laboratory conditions. Nonetheless it is a property whose response can be very different as a consequence of fire and is not only dependent on temperature but many other factors (Mataix-Solera et al., 2014).

GRSP, to the author’s knowledge, has never been used as an indicator of fire severity. So, as a novelty, its possible usefulness will be discussed. Changes in GRSP were detected at low temperatures, so it could be useful in low severity wildfires. However, its response to temperature was very dependent of the soil texture. AMF response to fire is not predictable (AMF can increase, decrease or disappear) and is extremely dependent on fire influence in the host-plants (Hart et al., 2005). Measuring GRSP immediately after wildfire is not directly related with fire impact on AMF community. GRSP will not be affected by external inputs related to the fire. Its stocks in soil depend on production and decomposition rates (Rillig, 2004) and are only extractable after hyphae die and decompose. Thus, GRSP results will only provide information about heating, not about the AMF community. Further studies after wildfires would be required to support its usefulness under field conditions.

CONCLUSIONS

Glomalin Related Soil Protein content was clearly affected by heating. The variations in content were detected even at low temperatures. These variations were slightly different between soil textures. The response of GRSP content to temperature was dependent on soil properties. Soil samples were clustered by texture. The main factor defining the GRSP response to temperature was TCA. The use of GRSP as an indicator could provide useful information when others parameters fail, such as SOC (which can be affected by external inputs) or SWR (whose presence is not always guaranteed). Although using more than one parameter at the same time would be recommended. Comparing burnt and unburnt GRSP content might provide useful information about temperatures reached during a fire. We propose to include GRSP content as a fire severity indicator. Field studies about this topic would be required.

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CAPÍTULO 2

Sensitivity of glomalin-related soil protein to wildfires: immediate and medium-term changes

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ABSTRACT

Forest fires are part of many ecosystems, especially in the Mediterranean Basin. Depending on the fire severity, they can be a great disturbance, so it is of especial importance to know their impact on the ecosystem elements. In this study, we measured the sensitivity of glomalin related soil protein (GRSP), a glycoprotein produced by arbuscular mycorrhizal fungi (AMF), to fire perturbation. Two wildfire-affected areas in the SE Spain (Gata and Gorga) were studied. Soil organic carbon (SOC) was also measured. Effects on GRSP immediately after fire were analyzed in both areas, while in Gorga a monitoring of GRSP stocks over a year period after the fire was also carried out. Soil samplings were carried out every 4 months. Plots (1 x 2 m^2) were installed beneath pines and shrubs in burned and an adjacent control area. Results of GRSP content immediately after fire only showed significant differences for shrub plots (burned *vs* control) ($P < 0.01$) in the Gorga site. However, a year of monitoring showed significant fire effect on GRSP content in both plot types (pines and shrubs). Control plots varied considerably over time, while in burned plots GRSP content remained constant during the whole studied period. This research provides evidence of the sensitivity of GRSP to a wildfire perturbation.

Key words: glomalin related soil protein; arbuscular mycorrhizal fungi; soil organic carbon; fire severity

INTRODUCTION

Wildfire is a natural and ecological factor in many ecosystems. However, human influence on fire frequency and the modification of vegetation patterns have made them an environmental problem in some locations (Cerdà and Mataix-Solera, 2009). Independent of their origin, wildfires affect most soil physical, chemical and biological properties (Certini, 2005) and therefore, soil quality (Fernández et al., 1997). It is of relevant importance to get to know the fire impact, in the short-, medium- and long-term, on the soil plant system in order to take appropriate decisions about post-fire strategies or management.

Most research based on assessing the fire severity, focusses on studying soil chemical quality; changes in organic material, available macronutrients and nitrogen (Couto-Vázquez and González-Prieto, 2006; Fernández et al., 1997; Úbeda et al., 2005), soil water repellency (SWR) and aggregate stability (AS) (Mataix-Solera et al., 2011; Mataix-Solera and Doerr, 2004) or vegetation cover changes (Úbeda et al., 2006), but of special interest are those related to soil biology due to its sensitivity to perturbations (Bárcenas-Moreno et al., 2011a; Bárcenas-Moreno et al., 2011b).

In forest environments, common disturbances such as fires can significantly alter soil biota (Mataix-Solera et al., 2009). The penetration of heat through the soil during a fire is one of the most important factors determining the severity of fire on the microbial and plant community. The fire itself affects microorganisms, due to the soil temperature reached during fires, and indirectly by changes in other soil properties and plant cover (Hart et al., 2005). After a fire, microorganisms are also influenced in the long-term by subsequent alterations in substrate availability, microclimate, and the presence of host plants especially for mycorrhizal fungi (Treseder et al., 2004). Changes in plant density and species composition might be expected to result in changes in the abundance and activity of mycorrhizal fungi that depend on those plants. At the same time, changes in soil chemistry and forest floor microclimate might affect the growth and activity of various species or groups of mycorrhizal fungi directly. Such changes will have subsequent effects on the performance of specific plant species and eventually on plant community structure (Hart et al., 2005). Decreases of 50% in the infection of Arbuscular Mycorrhizal Fungi (AMF) in burned soils have been observed (Wicklow-Howard, 1989).

AMF form mutualistic associations with about 70% of plant families (Newman and Reddell, 1987) and are abundant in all major terrestrial biomes (Treseder and Cross, 2006). Their presence tends to be higher in mineral soils with low organic content. These fungi produce the iron-containing, heat stable glycoprotein (extracted at 121°C) known as glomalin, which is contained within their hyphal and spores walls (Driver et al., 2005; Wright and Upadhyaya, 1996). As the hyphae senesce, they are thought to leave a residue of glomalin within the soil (Treseder and Turner, 2007). Operationally, glomalin extracted from soil is called glomalin-related soil protein (GRSP) (Rillig, 2004a). In the last years, it has been widely studied due to its implication in carbon and nitrogen storage (Treseder and Turner, 2007), and also for its role in AS, WR and sequestration of potentially toxic elements in soils (Cornejo et al., 2008; Lozano et al., 2013; Rillig, 2004b). These functions of GRSP could be especially important in semiarid areas, where GRSP could contribute to soil quality and help in the recovery of degraded areas (Bonfim et al., 2013). However, both its physiological and ecological functions remain unclear.

The protein has a relatively long soil life. Steinberg and Rillig (2003) found slow decomposition rates in laboratory incubations in Hawaiian soils. Pools of GRSP are responsive, even in the short-term, to ecosystem perturbations like warming (Rillig et al., 2002) and agricultural management practices (Wright and Anderson, 2000). Some authors have proposed GRSP as an indicator of soil health or recovery (Fokom et al., 2012; Rillig et al., 2003). However, most of the studies focus on agricultural practices or soil management.

GRSP response to heating has already been tested in a laboratory approach (Lozano et al., 2015). Based on our previous findings and GRSP sensitivity to perturbations, we think that GRSP content will be affected by wildfire over time. In this study, the GRSP response to wildfires under field conditions has been assessed. The main objectives of this work were: (i) to learn how the GRSP content in soil is directly affected by wildfires in two different soils and under different plant cover influence (*Pinu*s vs. shrubs), and (ii) to study the effects of wildfire on GRSP content across time. To achieve this, its evolution during a year after a wildfire was studied.

MATERIAL AND METHODS

Study areas and soil sampling

Soil samplings were carried out in two different forest sites in Alicante province (SE Spain): Gorga (38° 44' 56"N; 0° 2' 32"E) and Gata de Gorgos (38° 43’94" N; 0° 22’58’’ E). A characterization of soil properties is given in Table 1. Gata de Gorgos (Gata) site has a sub-humid Mediterranean climate type with a mean annual precipitation of 692 mm and mean temperature of 17.9°C. The soil is a Lithic Rhodoxeralf (Soil Survey Staff, 2014) developed over Cretaceous limestones. The soil texture is clay with 19% sand, 38% silt and 44% clay (Table 1). The vegetation area is primarily composed of *Pinus halepensis* M. and *Quercus coccifera* L. Gorga site has a sub-humid Mediterranean climate type with annual precipitation of 500 mm and mean temperature of 14.6°C.

Table 1: Main characteristic of the soil samples used (0-2.5 cm depth from the mineral soil horizon).

Site	AS (%)	TCA (%)	SOM (%)	pH	Carbonates (%)	Texture* (sand, silt, clay (%))
Gorga	84.1 (1.3)	76.4 (0.0)	4.2 (0.2)	7.9 (0.1)	57 (5)	Sandy silty loam (50, 41, 9)
Gata	91.0 (0.3)	80.8 (0.1)	9.3 (2.4)	7.8 (0.0)	48 (4)	Clay (19, 38, 44)

AS; aggregate stability, TCA; total content of aggregates, SOM; soil organic matter content. Mean values and standard deviation in brackets. *; Sand (2-0.05), Silt (0.05-0.002), Clay (< 0.002). Standard deviation between parentheses.

The soil is a Lithic Xerorthent (Soil Survey Staff, 2014) developed over Jurassic limestones. The soil texture is loam with 50% sand 41% silt and a 9% clay (Table 1).

The tree stratus is mainly dominated by *Pinus halepensis* and shrub species primarily represented by *Rosmarinus officinalis* L., *Cistus sp*. and *Brachypodium* sp.

Forest fires affected both sites. Gorga fire took place in July 2011 and the affected area was 40 ha. Gata fire occurred in July 2012 and affected a total area of 39 ha.

Both sites were sampled immediately after fire. Samples were collected using as a factor, the type of vegetation cover; *P. halepensis* influence versus shrubs. Significant changes in soil after a wildfire are normally in the first top-soil centimeters due to the low thermal conductivity of the soil (Badía et al., 2014; Pattinson et al., 1999). Thus, soil samples were taken from the first 2.5 cm of the mineral topsoil A horizon. The sampling was done by selecting stems randomly. Twelve samples per site were collected; 6 (3 beneath pines and 3 beneath shrubs influence) in the burned area and 6 (3+3) in the unburned adjacent area with similar conditions used as controls. Each sample is composed of three subsamples taken under the same stem. Samples beneath pines were collected at 0.5 m of distance from the base of trunks, subsamples were separated 50 cm between them. Shrubs plots were similar in species composition, a mix between *R. officinalis* and *Braquypodium* sp. The Gorga site was also used as a monitoring site. Plots (1x2 m^2) for monitoring were installed in burned (B) and adjacent control (C) unburned area, underneath *P. halepensis* (P) and shrub (S) species. The plots were sampled immediately, 4, 8 and 12 months after the fire to measure the evolution of some soil parameters in the different seasons.

All samples were dried at room temperature (20 - 25°C) to a constant weight and sieved (< 2 mm) to eliminate coarse soil particles before soil analysis

Soil parameters analyzed

Characterization of soils was carried out on air-dried samples (Table 1), which included pH (1:2.5 w/v, distilled water), texture determined by Bouyoucos method (Gee and Bauder, 1986), soil organic carbon (SOC) determined by potassium dichromate oxidation (Nelson and Sommers, 1982) and AS and total content of aggregates, by the rainfall simulator method of Roldán et al. (1994); a more detailed description of this last methodology can be consulted in Chrenková et al. (2014).

GRSP extracted from soil was the easily extractable glomalin (EEG), which corresponds with the last fraction deposited into the soil. GRSP was extracted from 0.25 g subsamples with 2 ml citric acid buffer, pH 7.0 at 121°C for 30 min in autoclave. After extractions, samples were centrifuged at 3000 × during 15’ to remove soil particles. Protein in the supernatant was determined by a Bradford assay (Wright and Upadhyaya, 1996).

Statistical analysis

Normality and homogeneity of variances for all data were tested. One-way ANOVA analyses were done to evaluate the immediate and medium-term post-fire effects on GRSP content. Immediately after fire, the differences in GRSP and SOC content between B and C plots were analyzed.

Evolution of GRSP content over time was evaluated as follow: firstly the differences in GRSP content between plot types at each sampling time were calculated, an ANOVA per soil sampling was made, and secondly, the fire effect over time was studied on each plot type, an ANOVA was made per plot type.

The separation of means was made according to Tukey’s honestly significant difference at $P < 0.05$.

Pearson’s correlation coefficients (r) were used to quantify the linear relationship between SOC and GRSP. Statistical analyses were performed using the SPSS 11.5 package (© SPSS Inc, 1989).

RESULTS

Direct effects of fire

Fire influence in GRSP was different between sites (fig 1). Results did not show any direct effect of fire on GRSP content in Gata site ($P > 0.05$; fig. 1a). However, in Gorga site, significant differences between GRSP content were found ($P < 0.05$). Tukey test showed two groups: 1) BP (burned pine), CP (control pine), CS (control shrubs) and 2) BS (burned shrubs) (fig 1b). The fire produced a significant decrease in GRSP in BS plots (around 50% less compared to CS; fig. 1b).

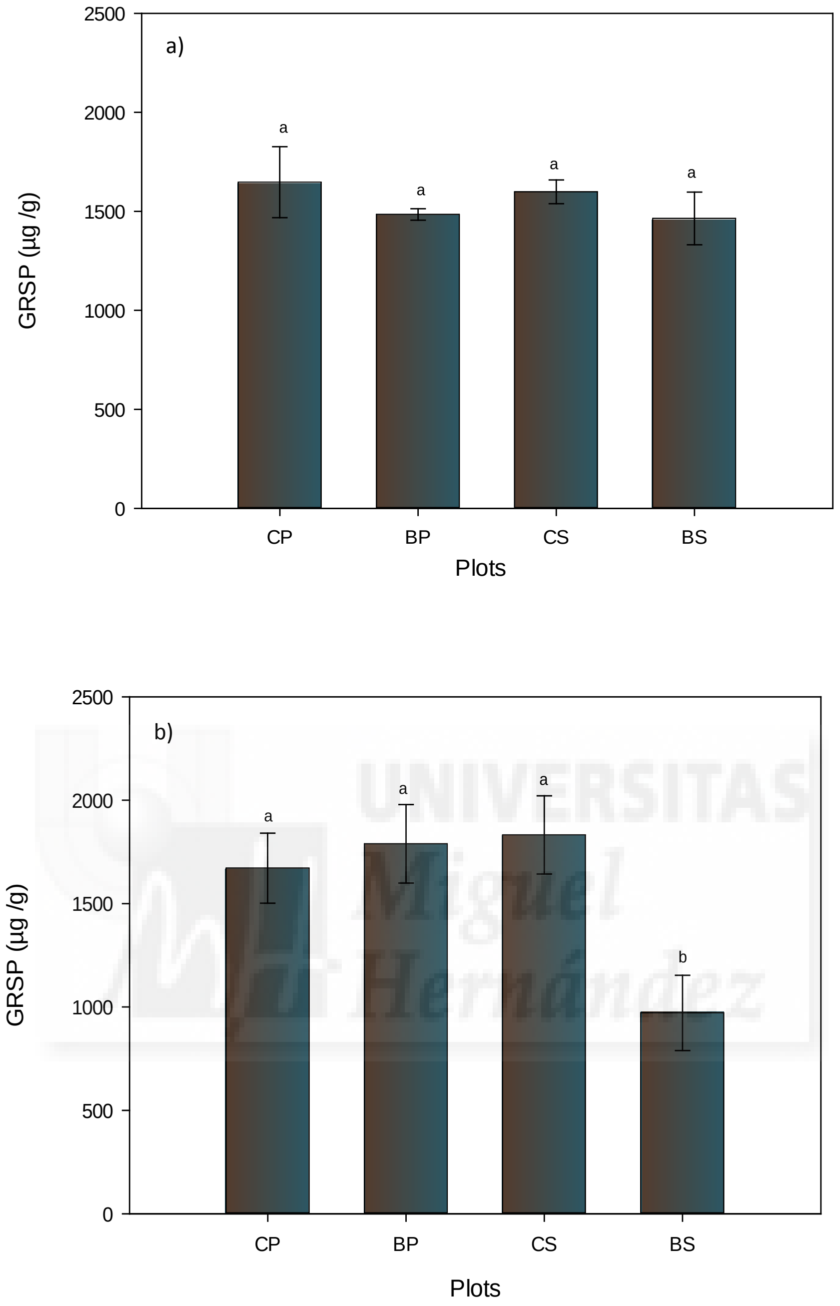

Figure 1: (a) Glomalin-related soil protein (GRSP) mean content (µg /g) immediately after fire of each type plot in Gata site. (b) GRSP mean content (µg /g) immediately after fire of each type plot in Gorga site. CP= control pine; BP= burned pine; CS= control shrubs; BS= burned shrubs. Standard errors in bars. Different letters show the statistically significant differences between the different GRSP content determined with the Tukey test ($P \leq 0.05$).

The effect of fire on SOC content was similar in both sites. Significant differences between controls and burned samples in SOC content were only found under shrubs (fig.2).

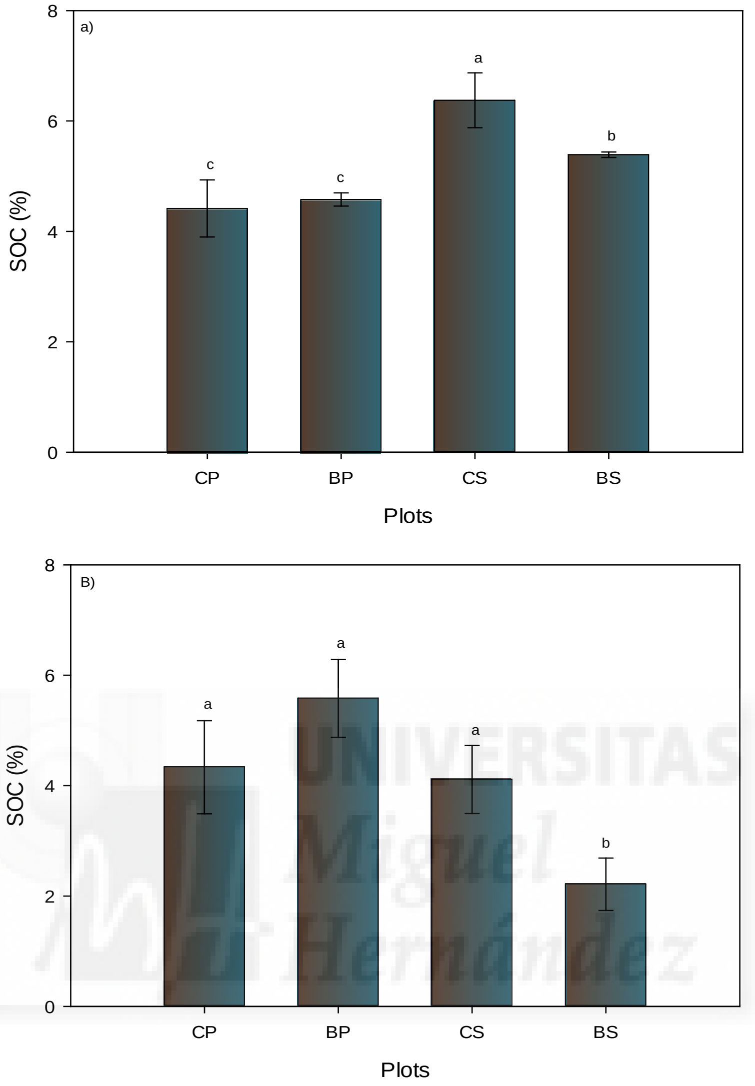

Figure 2: (a) Soil organic carbon (SOC) mean content (%) immediately after fire of each plot type in Gata site. (b) SOC mean content (%) immediately after fire of each type plot in Gorga site. CP= control pine; BP= burned pine; CS= control shrubs; BS= burned shrubs. Standard errors in bars. Different letters show the statistically significant differences between the different SOC content determined with the Tukey test (P ≤ 0.05).

Effects in GRSP stocks over a year period after the wildfire (Gorga site)

Soil samplings corresponding to December 2011, March and July 2012 were analyzed individually. Significant differences in GRSP content between plots (fig 3 capital letters) were observed in all of them. Tukey test classified the GRSP content in three groups: (1) BP, (2) BS and (3) the CP and CS plots.

When the particular evolution over time of each plot type (CP, BP, CS and BS) was analyzed, significant differences were only observed in Control plots (CS and CP) (fig.3, lower cases). Control plots showed the highest values in July (2011 and 2012) and the lowest in December (fig. 3). These peaks were inversely related to average rainfall recorded for the same months of sampling (fig. 3). No significant changes over time were found in burned plots (BP and BS) for GRSP contents. The monitoring in burned plots revealed strong differences in GRSP content between BP and BS. Nonetheless, the behavior over time was similar for both types of vegetation, showing a static situation in the fire-affected area in contrast to the dynamic behavior found in the Control plots, particularly in the first months following the fire (fig. 3).

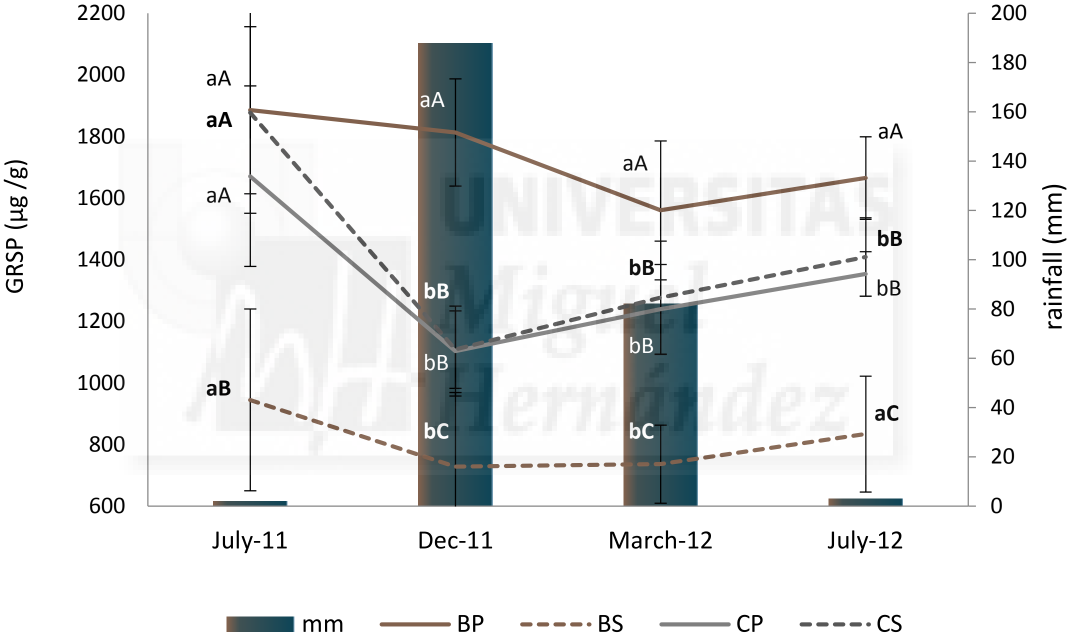

Figure 3: Temporal evolution of glomalin-related soil protein (GRSP; lines) and Rainfalls (mm; bars). CP= control pine; BP= burnt pine; CS= control shrubs; BS= burnt shrubs. Standard deviations in bars. Lower case represent differences between soil samplings per plot type and capital letters are referred to differences between plots types in each soil sampling.

Pearson correlations between SOC and GRSP for each plot type over the whole period were significant. These correlations were higher in control plots (fig. 4)

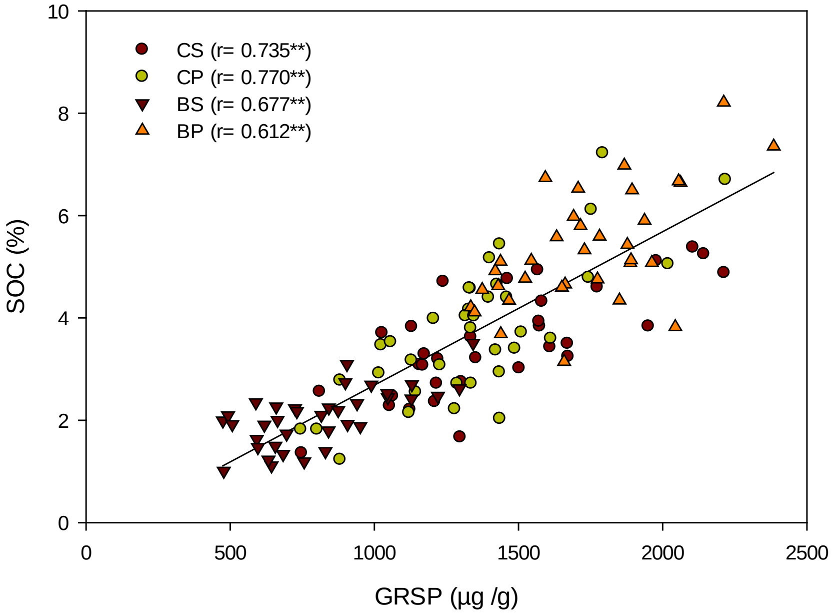

Figure 4: Relationship between glomalin-related soil protein (GRSP) content (µg /g) and soil organic carbon (SOC) content (%) over a year period time. r= Pearson's correlation coefficients. CP= control pine; BP= burned pine; CS= control shrubs; BS= burned shrubs.

DISCUSSION

Direct effects of fire

GRSP content is affected by heating. How GRSP content is affected will depend not only on temperatures reached during the fire and time of residence of heat, but also on initial soil characteristics such as soil aggregation, texture and SOC (Lozano et al., 2015).

No significant changes in GRSP content between burned and control samples in Gata (P and S) and Gorga Pine plots indicate that the fire did not reach very high temperatures (fig. 1).In a previous study, Lozano et al. (2015) observed that GRSP content decreased with temperature when a threshold of temperatures were reached. These temperature thresholds varied between soil types. In particular, in the cases of the soils of this study, significant decreases of GRSP in Gorga and Gata sites started at 250 and 300°C respectively. Below those temperatures, GRSP content did not significantly change in those soils. Therefore, differences in GRSP content between BS and CS plots in Gorga would be related with a higher fire severity. This is not strange if it is considered that temperatures reached in soil during a fire are very variable and mostly influenced by spatial distribution of the vegetation (Gimeno-García et al., 2004). In general, and under normal quantities of fuels, the range of maximum temperatures in shrublands (between 300-700°C) tends to be higher than in mature tree forest (200- 300°C Neary et al., 1999), which would explain the differences in GRSP content found between burned plots (pine *vs* shrub) found in the Gorga site. SOC results also corroborate these assumptions. The effect of heating on SOC has been widely studied (Badía et al., 2014, Certini et al., 2011, González-Pérez et al., 2006). Normally, SOC significantly decreased around 250°C (Chandler et al., 1983, Fernández et al., 1997, Terefe et al., 2008), although depending on many factors, in a wildfire, SOC can be changed from almost total destruction to an increase as consequence of external inputs coming from necromass and semi-pyrolized ash (Fernandez et al., 1997, Jordán et al., 2011). In this case we checked under laboratory conditions, changes in SOC in Gata and Gorga soil which started at over 180 and 200°C respectively (Lozano et al., 2015). Thus, no significant changes in Gata and Gorga pine plots are indicative of a low fire severity. On the contrary, differences in shrubs plots would be due to a higher fire severity, at least in Gorga. In a previous study under laboratory controlled burning we observed a different behavior between GRSP and SOC in Gata soil (Lozano et al., 2015). This could explain the observed differences between GRSP and SOC response to fire in Gata BS plots.

Effects in GRSP stocks over a year period after the wildfire (Gorga site)

Very little research has been done to study how GRSP stocks are affected by fire. Treseder et al. (2004) studied GRSP content evolution in areas previously affected by fire 3, 15 and 45 years in Alaskan boreal forest. A peak of GRSP content 15 years after fire was found. The increase would be associated with the proliferation of AMF in earlier succession. Knorr et al. (2003) did not find direct evidence for an effect of low intensity prescribed fire on GRSP content one year after in an oak tree area. The low intensity and severity of fire would be the main reason. Controlled fires are usually of low intensity and severity and just consume the understory vegetation and part of the forest floor layers, but do not damage trees (Pereira et al., 2011; Úbeda et al., 2005). However, according to our results, just the analyses of the changes after a year (without monitoring) would not always be enough to determine a possible influence of fire on GRSP content. CP and BP plots were similar in GRSP content immediately and a year later after fire, however the evolution over time was significantly different between burned and control plots (fig. 3). Obversely, in the case of shrub plots, the influence of fire on GRSP content was evident immediately and a year after the fire.

GRSP content in burned plots did not show changes over time. This lack of variation in burned plots over the period studied contrasts with the seasonal variations observed in the control plots, suggesting medium-term influence of fire on GRSP dynamics, at least during the first year after the fire (fig. 3). Treseder et al. (2004) also observed medium term effects in GRSP content, but contrary to us, they observed an increase in GRSP two years later after a fire. That was attributed to the lack of glomalin mineralization due to a loss or delay in the recovery of decomposers. The lack of variation found suggests no production and no decomposition at least at the same levels at control plots. Gorga fire was quite severe and destroyed all the vegetation. The loss of host plants directly affects mycorrhizal fungi (Neary et al., 1999) and stops the allocation of organic C to AMF and as a consequence, there is no C to invest in glomalin production.

This study highlights that glomalin production and it deposition into the soil (GRSP) are extremely dependent on the season. Lutgen et al. (2003) observed seasonal influence on GRSP stocks in temperate grassland, although the content was not correlated with soil moisture. Bellgard (1993) suggested that seasonal patterns in the AM colonization were related to the increase in temperatures. This seasonal behavior is completely normal from the point of view that glomalin is produced by AMF, so GRSP stocks may also depend on the same factors that control AM growth (Treseder and Turner, 2007). In this study, there are at least two reasons that would explain this variability in the stocks. Firstly, during autumn and winter photosynthesis is lower, so there is a decrease in SOC production, and in turn, a decrease in allocation to fungi (Harris and Paul, 1987, Harris et al., 1985; Johnson et al., 2003). Secondly, glomalin is linked to stress-related proteins (Gadkar and Rillig, 2006), so it might be possible that strong stress caused by drought during summer season may cause overexpression of this protein. In fact, host plants invest more in mycorrhizal fungi during water stress periods (Auge, 2001).

GRSP and SOC relationship has been reported in previous works (Lozano et al., 2013, Rillig et al., 2003). An increase of GRSP has been related with increasing in SOC in seasonal studies (Gispert et al., 2013) probably because GRSP is thought to contribute in the long-term to C storage (Lovelock et al., 2004). Results revealed that correlation coefficients corresponding to B plots were lower than the control ones. Different responses to temperature between SOC and GRSP have already been reported (Lozano et al., 2015), so fire influences this relationship. Changes in SOC quality due to fire could also be related to differences observed by Almendros and González-Vila (2012). Rillig et al. (2003) demonstrated the sensitivity of GRSP content to land use changes. They attributed the observed differences in GRSP decomposition rates between forests, afforested agricultural and agricultural soils to changes in the quality of SOC.

CONCLUSIONS

This study provides evidence that GRSP stocks are sensitive to perturbation such as a forest fire and emphasizes the importance of monitoring changes over time after wildfires. The direct influence of fire was not always clear, but it was over time. The study of GRSP together with SOC can provide useful information about fire severity.

GRSP content in control plots varied depending on the season of the year, while burned plots did not vary over the period of a year after the wildfire.

Fire affects AMF dynamics, which could be essential for ecosystem recovery. The study of soil biology is especially important due to its sensitivity to perturbation. Longer field studies about GRSP evolution over time, together with other indicators of soil and plant activity could be useful in monitoring soil recovery after a wildfire, and evaluate the use of GRSP as an indicator of soil health and recovery.

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CAPÍTULO 3

Biological and chemical factors controlling the patchy distribution of soil water repellency among plant species in a Mediterranean semiarid forest

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ABSTRACT

Natural soil water repellency is a property that has already been observed in forest soils and is characterized by its patchy distribution. There are many factors involved in its development. In this work, we have studied a large number of chemical and biological factors under the influence of different plant species (*Pinus halepensis, Quercus rotundifolia, Cistus albidus* and *Rosmarinus officinalis*) to learn which has the greatest responsibility for its presence and persistence in the top-soil layer. We observed strong and significant correlations between ergosterol, glomalin related soil protein (GRSP), extractable lipids, soil organic matter (SOM) content and water repellency (WR). Our results suggested lipid fraction as the principal factor. Moreover, apart from *Pinus,* fungal biomass seems to be also related to the SOM content. Soil WR found under *Pinus* appears to be the most influenced by fungi. Quality of SOM, to be precise, lipid fraction could be responsible for WR and its relationship with fungal activity.

Key words: water repellency, organic matter content, lipid fraction, glomalin related soil protein, ergosterol.

INTRODUCTION

Soil water repellency (SWR) has been observed in forest soils under different climatic conditions, soil types and vegetation covers (Doerr et al., 2000). SWR is normally characterized by a high spatial variability in persistence, with wettable and water repellent patches next to each other. This phenomenon is of special interest in semiarid areas, such as Mediterranean ecosystems, where water is considered to be one of the fundamental controls affecting the structure, function, and diversity of ecosystems (Rodriguez-Iturbe, 2000). In ecosystems where water resources are limited, even slight WR may play an important role in the infiltration patterns and the spatial distribution of water in the soil (Mataix-Solera et al., 2007). WR has hydrological impacts, but also ecological consequences, with repercussions on plant growth (Doerr et al., 2000). This could be the reason why several studies single out the production of hydrophobins by plants, as a possible ecological strategy (Mataix-Solera et al., 2007). It is thought to be a mechanism for improving water conservation by channeling water deep into the soil profile following preferential flow pathways (Moore and Blackwell, 1998; Robinson et al., 2010 and Scott, 1992), while at the same time reducing evaporation due to the spatial dryness of the surface layer (Doerr et al., 2000).

It has been proposed that the origin of natural WR is caused by organic compounds released from different plant species and sources, due to resins, waxes and other organic substances in their tissues. In the Mediterranean areas different evergreen trees (such as *Pinus* and *Quercus*) and shrubs are usually associated with SWR under natural conditions (Arcenegui et al., 2008; Jordán-López et al., 2008; Mataix-Solera et al., 2007; Verheijen and Cammeraat, 2007). There is a large quantity of research publications that associate SWR with the SOM content (Doer et al., 2000; Mataix-Solera and Doerr, 2004; Zavala et al., 2009). Even though, many of them suggest that this relationship could be due to the quality of SOM (Mataix-Solera et al., 2007; Rumpel et al., 2004). In fact, literature has emphasized the importance of lipid fractions released to soil by plants or microorganisms (fungi) (Franco et al., 2000; Hudson et al., 1994; Ma'shum et al., 1988), as well as the behaviour of specific characteristics of the organic matter, in general associated with moisture regimes, e.g., temporarily waterlogged soils (Fridland, 1982). In particular, considerable experimental effort has been carried out in the last decade to identify specific substances with a potential relevance on WR (De Blas et al., 2010; Doerr et al., 2005b; Franco et al., 1994, 1995; Hudson et al., 1994; McIntosh and Horne, 1994; Walis et al., 1993).

On the other hand, research highlights the point that the relationship between WR and plants may not always be direct: a group of fungi and microorganisms, which might be associated with specific plants, could also contribute to soil hydrophobicity through their products or by processing organic material (Feeney et al., 2004; Hallet and Young, 1999; Morales et al., 2010; White et al., 2000). In concrete, fungal hyphae, glomalin related soil protein and more recently ergosterol are being studied to understand their influence on the development of SWR (Rillig, 2005, Rillig et al., 2010; Young et al., 2012). GRSP is a glycoprotein produced primarily by arbuscular mycorrhizae (AM) (Buyer et al., 2011; Treseder and Turner, 2007). Glomalin is not exuded by AM hyphae, but is instead contained within hyphal walls (Driver et al., 2005). When the AM hyphae die and decompose, they are thought to leave a residue of glomalin in the soil (Treseder and Allen, 2000). The importance of the presence of GRSP relates to its supposed hydrophobic properties. Results on the influence of GRSP differ, so that the question is still unclear (Feeney et al., 2004; Young et al., 2012). Ergosterol is a specific component of fungal membranes and the major sterol in most filamentous fungi (Vanden Boosche, 1990). It is recognized as being an important biomolecule through which reduced permeability may occur in a wide variety of biological surfaces/membranes (Young et al., 2012). Its content is considered as a marker for living fungi and a good estimate of metabolically active fungal mycelium in soil (Montgomery et al., 2000).

In this research, we have studied at the same time chemical and biological factors involved in the occurrence of superficial SWR under different plant cover. Our aim here is to find out about which factors are the most relevant in the development of SWR and possible relationships between them. This research could be a contribution to better understanding of why this phenomenon occurs in the semi-arid Mediterranean context under natural conditions.

MATERIAL AND METHODS

Study area

The study area is located in the 'Sierra de la Taja' (38°23'N; 0°59'W) near Pinoso, in the province of Alicante (SE of Spain). The region has a semi-arid Mediterranean climate with a mean annual precipitation of 277.5 mm and a mean annual temperature of 15.8°C ranging from 7.8°C in January to 24.1°C in August (average 1980-2010). The whole area of the 'Sierra de la Taja' is approximately 500 ha. The samples were taken under similar conditions with respect to soil type, geology, plant distribution and slope. The soil is a Lithic Xerorthent (Soil Survey Staff, 2014), developed over Jurassic limestone. The soil texture in the area is loam with a 36% of sand, a 49% of silt and a 15% of clay.

The tree stratus of the area is formed by *Pinus halepensis* Miller of approximately 40 years and *Quercus rotundifonlia* is also present. Shrub vegetation comprises mainly *Quercus coccifera* L., *Rosmarinus officinalis* L., *Juniperus oxycedrus* L., *Cistus albidus* L., *Brachypodium retusum* Pers. (Beauv.), *Stipa tenaccissima* L., and *Pistacia lentiscus* L. Tree and shrub species are mixed in the study area, but as a consequence of the relatively low density of vegetation, it was possible to carry out the sampling in microsites per stem of each species, avoiding interference between them.

Soil sampling

Samples were taken in September 2011, when the SWR is expected to be at its strongest after the typical Mediterranean summer drought (DeBano, 1981; Dekker and Ritsema, 1994; Doerr et al., 2000). Soil samples were collected from the first 2.5 cm of the mineral A horizon at microsites beneath each of the four most representative species (*Pinus halepensis, Rosmarinus officinalis, Quercus rotundifolia* and *Cistus albidus*; n=15 per species) and 5 samples from bare soil with no influence from any species. The sampling was done by selecting stems randomly, and taking two samples per stem. Half of samples were preserved and frozen at -5°C and the half were preserved at 25°C. The distance between the stems sampled was around 10 m.

Laboratory methods

Soil samples (not frozen) were dried at room temperature (20 - 25°C) to a constant weight and sieved (2 mm) to eliminate coarse soil particles before soil analysis. Soil pH was measured in aqueous soil extract in de-ionised water (1:2.5 w:s) at 25°C. SOM content was analysed by rapid dichromate oxidation of organic carbon (Walkley and Black, 1934).

For measuring WR, approximately 15 g of soil per sample were placed on separate 50-mm diameter plastic dishes and exposed to a controlled laboratory atmosphere (20°C, ~50% relative humidity) for one week to eliminate potential effects of any variations in preceding atmospheric humidity on SWR and in accordance with the findings of Doerr et al. (2005a). The persistence of WR was measured by the Water Drop Penetration Time (WDPT) test (Wessel, 1988). This involved placing 3 drops of distilled water (~ 0.05 ml) onto the sample surface and recording the times required for their complete penetration. The average time for triplicate drops has been taken as the WDPT value of a sample. Penetration times were classified in intervals and in classes according to Bisdom et al. (1993), with WDPT ≤ 5 s representing wettable and WDPT> 5 s water repellent conditions. The logarithm of the WDPT value in seconds has been used; being water repellent if the value of log (WDPT) is > 0.7. The water repellency classes used are indicated in the Table 1.

Table 1: WDPT classes and class increments used in the present study (after Bisdom et al. 1993).

Repellency rating	Wettable	Water repellency								
		slight			strong			severe		extreme
WDPT classes	≤ 5	10	30	60	180	300	600	900	3600	> 3600
WDPT interval (s)	≤ 5	6-10	11-30	31-60	61-180	181-300	301-600	601-900	901-3600	> 3600
Log WDPT interval	≤ 0.7	0.7-1.0	1.0-1.5	1.5-1.8	1.8-2.3	2.3-2.5	2.5-2.8	2.8-3.0	3.0-3.6	> 3.6

Extractable lipids were Soxhlet-extracted from soil samples (10 g) with a dichloromethane–methanol (3:1 v/v) for 16 h at 70°C (González-Vila et al., 2003; Van Bergen et al., 1997). Extracts were filtered and dried and then total lipid content was gravimetrically determined and referred to as percentages of g soil.

To determine the possible relationship of fungal activity and its presence in soil with WR, three different fungal parameters were measured; GRSP, mycelium length and ergosterol. GRSP measured was the Easily Extractable Glomalin, which corresponded with the fraction of protein most recently deposited into the soil. GRSP was extracted from 0.25 g subsamples with 2 ml citric acid buffer, pH 7.0 at 121°C for 30 min. After extractions, samples were centrifuged at 3000 × during 15’ to remove soil particles. Protein in the supernatant was determined by a Bradford assay (Wright and Upadhyaya, 1996). Concentrations of glomalin were extrapolated to µg/g by correcting for the dry weight of coarse fragments (> 0.25 g) included in the extraction of soil.

For the measurement of mycelium length, hyphae were extracted from a 10 g soil subsample by an aqueous extraction and membrane filter technique (Bååth and Söderstrom, 1980; Bardgett, 1991; Hanssen et al., 1974). Soil samples were mixed and suspended in 100 mL of deionized water. Suspensions diluted (10^{-2} ml) for measuring the mycelium length were stained with 0.05% Trypan Blue, filtered (1.2 µm Millipore cellulose membranes) and transferred to microscope slides. The mycelium length was measured microscopically at 40X.

Ergosterol extraction was done according to the method described by Rousk and Bååth (2007) to estimate metabolically active fungal mycelium. 1 g dry weight of soil was transferred to test-tubes and then ergosterol was extracted in 5 ml 10% KOH in methanol, sonicated for 15 min followed by 90 min heat treatment at 70°C, and partitioned twice with 2 ml cyclohexane. The combined cyclohexane phases were evaporated to dryness at 40°C under N_2. The samples were then dissolved in 500 µl methanol, heated at 40°C for 15 min, filtered through a 0.45 µm filter, and analyzed using HPLC with a UV detector (282 nm).

Statistical analysis

Normality and homogeneity of variances for all data were tested, and log transform was made when necessary. ANOVA-one way analysis was done to calculate differences between species. Pearson’s correlation coefficients (r) were calculated to quantify the linear relationship between parameters. Partial correlations were assessed to investigate possible influences of SOM content in the relationship between WR and pH, and also in the WR and GRSP and ergosterol. Statistical analyses were performed using the SPSS 11.5 package (© SPSS Inc, 1989).

RESULTS

Soil water repellency distribution under different plant species and its relationship with SOM content

Our results of the WDPT showed that the 41% of the total samples were hydrophobic. The majority of water repellent samples were classified as slight or strongly water repellent (around 22% and 14%, respectively), while severe WR was only detected in 5% of samples. No extreme water repellent sample was found. On the other hand, the occurrence of WR was different between species and was higher under *Pinus* (87% of samples) and *Quercus* (60% of samples). In *Rosmarinus* samples only 28% of the samples were repellent. All *Cistus* and bare soil samples were wettable. In spite of the high number of water repellent samples found under *Pinus*, the strongest persistences were found under *Quercus* (fig. 1).

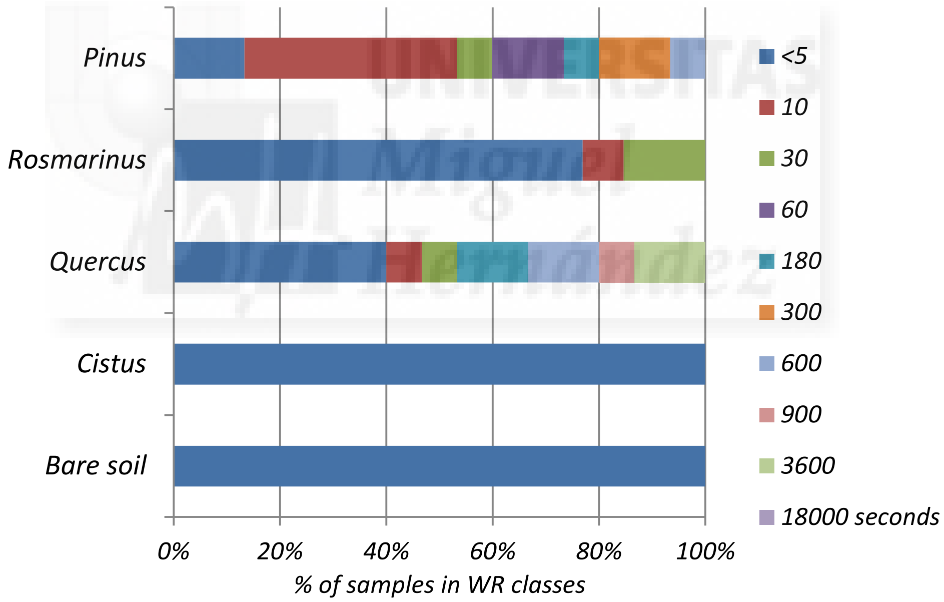

Figure 1: Relative frequency of water repellency classes beneath the different species and bare soil (n=64).

SOM content revealed significant differences between soils under different plant species (fig. 2), being higher in samples of *Pinus* and *Quercus*, with mean values of 12.98% and 12.93% respectively.

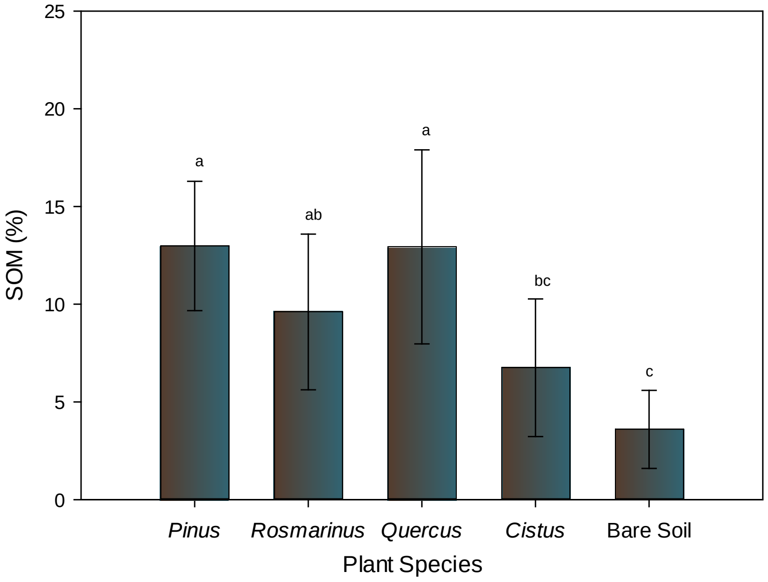

Figure 2: SOM content beneath the different species and bare soil (standard deviations in bars). Different letters show the statistical significantly differences between the different species and bare soil determinated with the Tukey test ($P \leq 0.05$).

Positive significant correlations were found between WR and SOM content pooling the data of all species together (r= 0.867**) and separately for the species that showed samples with WR (Table 2 and fig. 3), with the best correlations for *Pinus* and *Quercus* (r= 0.855**, r= 0.934** respectively) (fig. 3).

Table 2: Pearson's correlations coefficients between WR and soil parameters for each specie.

	Species	SOM	pH	pH partial (SOM)[a]
WR (log WDPT)	*Pinus*	0.855**	-0.763**	-0.552
	Rosmarinus	0.776**	-0.833**	-0.766
	Quercus	0.934**	-0.373	n.c.[b]
	Cistus	n.c. [c]	n.c. [c]	n.c. [c]

[a] Coefficient of partial correlations between GRSP and WR (log WDPT) controlling the effect of SOM.
[b] n.c.: not calculated because of the lack of SWR under this plant species and bare soil.
c n.c.: not calculated because of the absence of SWR under this plant species.
***,**,*; significant at $P \leq 0.001$, 0.01, 0.05 respectively.

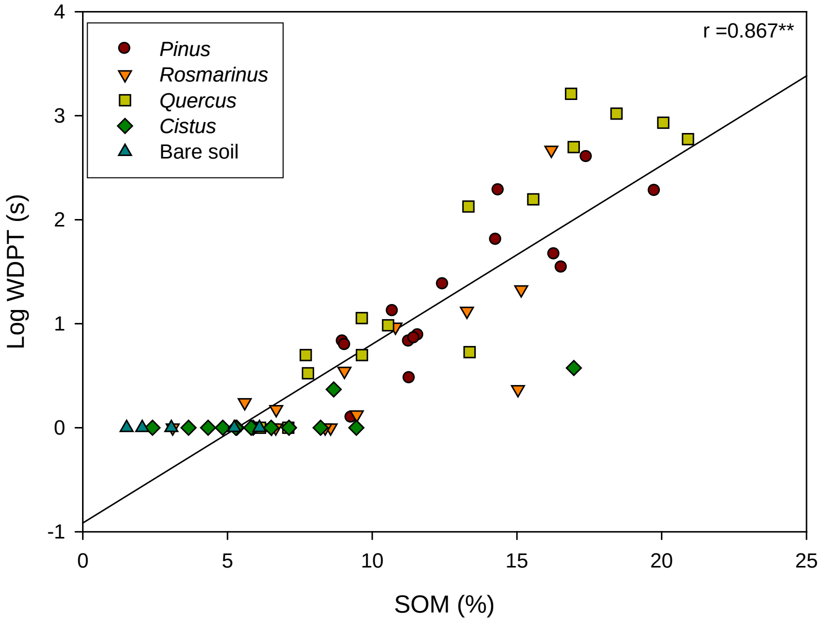

Figure 3: Relationship between SOM content and WR (log WDPT) of samples taken beneath the different species and bare soil (r= Pearson's correlation coefficient).

Extractable lipid's content

For this analysis, samples from eighteen soils under *Pinus* (n= 8) and *Quercus* (n= 10) very similar in SOM content but quite different in WR were selected to perform lipid extractions in order to check the role of the quality of SOM content in soil WR (fig. 4).

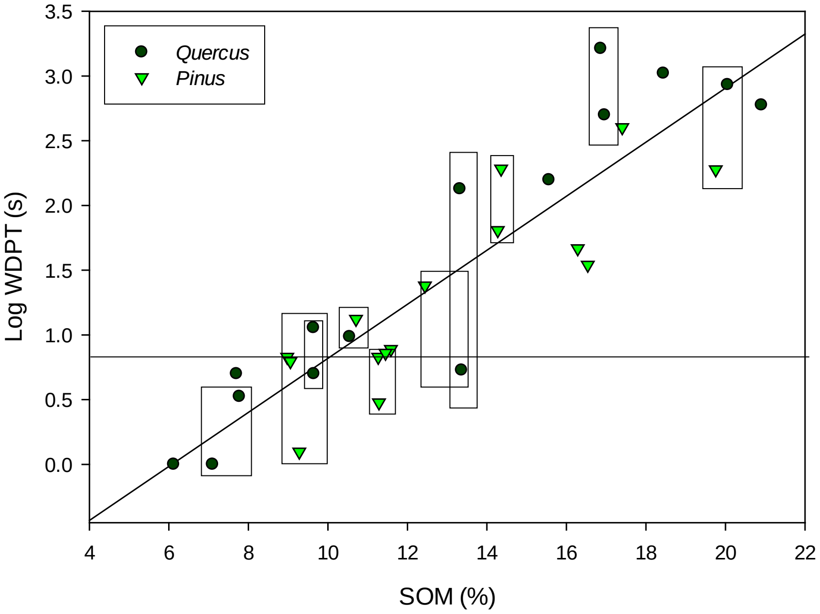

Figure 4: Relationship between WR (log WDPT) and SOM content of soil samples beneath Quercus and Pinus. Rectangles show samples selected with similar SOM content and different WR for extractable lipids analysis.

The concentrations of extractable lipids in the soils were 0.12 ± 0.09% for *Pinus* and 0.12 ± 0.10% for *Quercus* and achieved maximum values in repellent samples (fig. 5). In fact, extractable lipid's content showed a close relationship with log WDPT (r= 0.858**; figures 5 and 6). The Pearson's correlation coefficients were also significantly high if analyzed per species (*Pinus*; r= 0.753 and *Quercus* r= 0.953**; fig. 6).

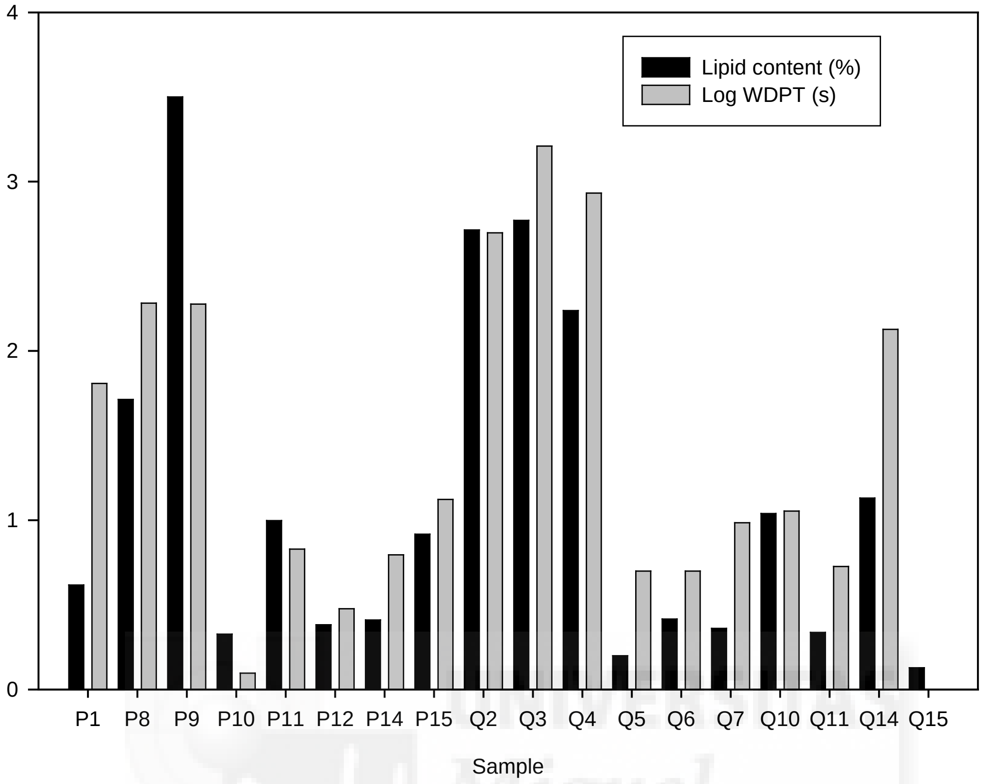

Figure 5: WR and extractable lipid content for selected samples studied. (P= *Pinus* and Q= *Quercus*, numbers correspond with the name of sample).

Water repellency persistence and pH

For all four species, pH values of water repellent samples were generally lower than those of wettable samples. The pH measured followed this order *Pinus* = *Quercus* < *Rosmarinus* < *Cistus* < bare soil. We found negative correlations with WR, although these correlations seem to be related to SOM content (fig. 7) as the partial correlation showed (Table 2). In concrete, in samples under *Pinus*, that correlation was highly significant (r= -0.763**), but disappears in the partial correlations (r= -0.552) indicating that it is an apparent correlation really controlled by SOM content. In contrast, samples under *Quercus*, in spite of having the same pH mean values as *Pinus*, had no correlation between these parameters (Table 2).

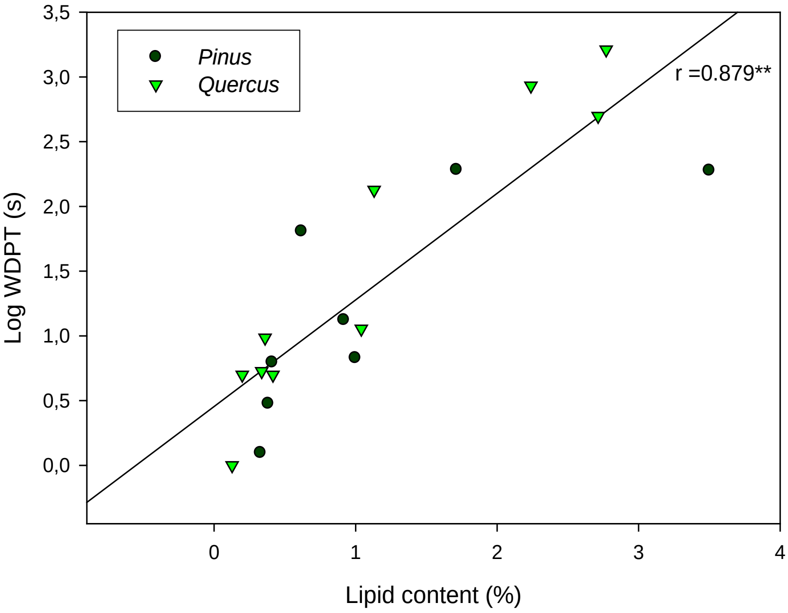

Figure 6: Relationship between extractable lipid contents and WR (log WDPT) for the selected samples studied.

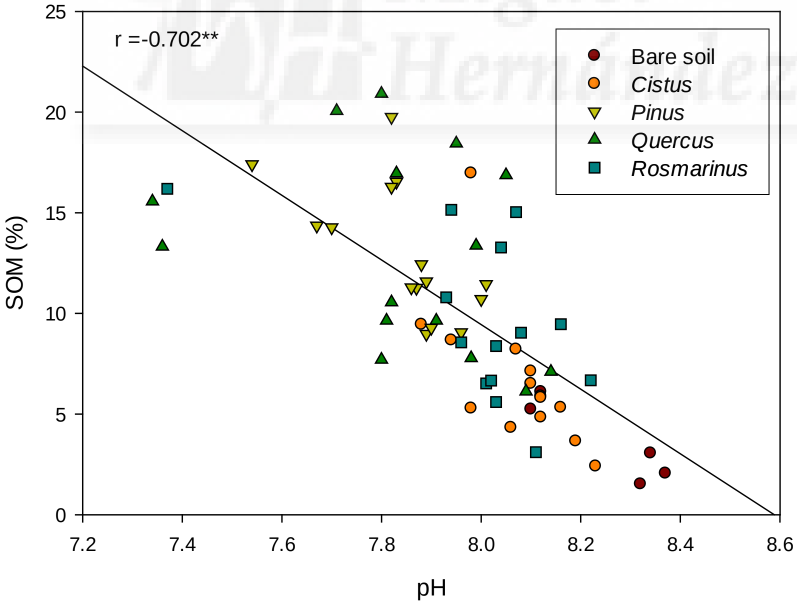

Figure 7: Relationship between pH and SOM content of samples taken beneath the different species and bare soil.

Fungal related parameters: GRSP, Mycelium length and Ergosterol

GRSP from AM fungi revealed significant differences between species (fig. 8). The highest average content of GRSP was found in *Rosmarinus* (fig. 8).

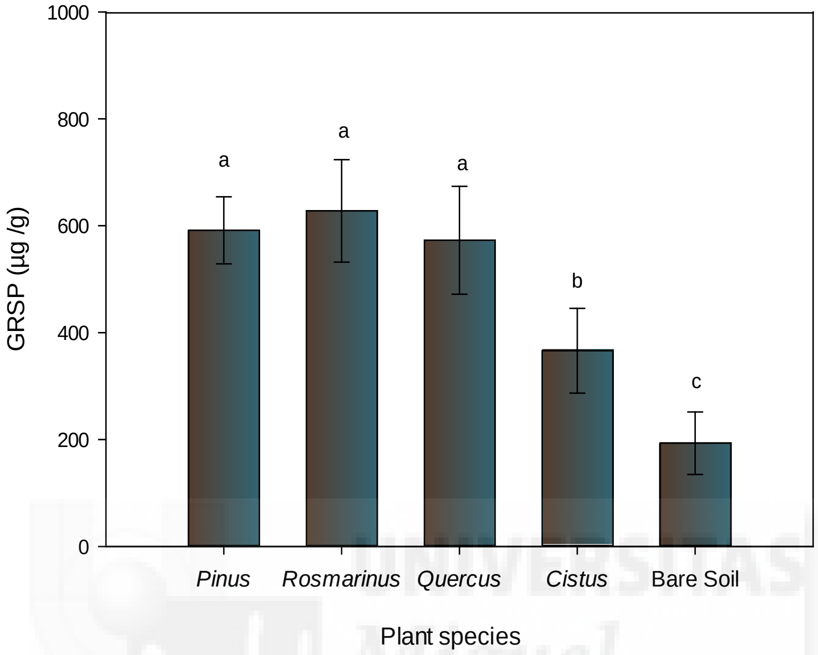

Figure 8: GRSP content beneath the different species and bare soil (standard deviations in bars). Different letters show the statistically significant differences between the different species and bare soil with the Tukey test (P ≤ 0.05).

Between GRSP and SOM content, we found significant correlations except for *Pinus*, in spite of showing a clear tendency (fig. 9).

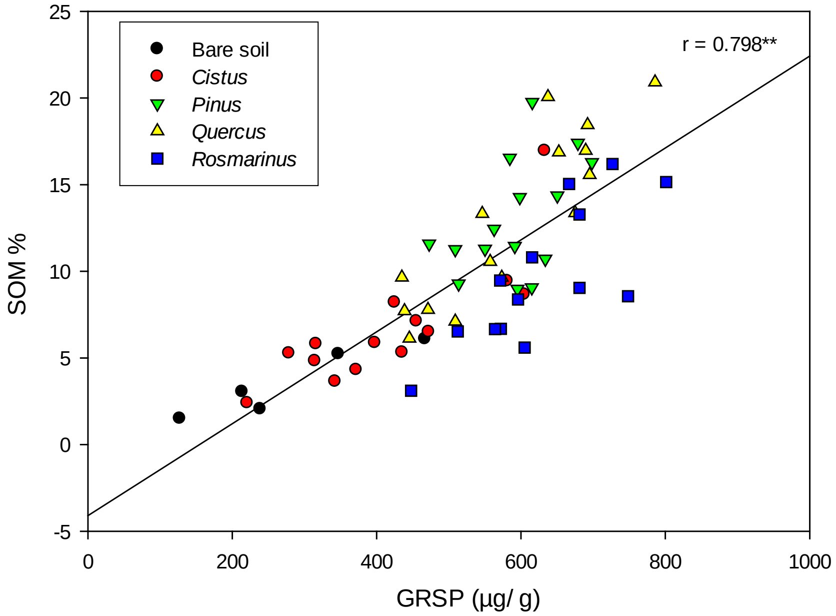

Figure 9: Relationship between GRSP and SOM content beneath the different species and bare soil.

These correlations were especially high for bare soil (r= 0.948*), *Quercus* (r= 0.868**) and *Cistus* (r= 0.895 **; Table 3).

Pearson's correlations were also significant between WR and GRSP (Table 3). The higher correlations corresponded to samples of *Quercus* (r= 0.763**). *Cistus* and bare soil were not calculated because there were no water repellent samples in those groups (Table 3). Nevertheless, this relationship seems to be controlled by SOM as partial correlations indicate, with the exception of *Pinus* (Table 3).

Table 3: Pearson's correlation coefficients between GRSP and soil parameters for each species.

	Species	SOM	WR (log WDPT)	WR partial (SOM)[a]
GRSP	*Pinus*	0.491	0.674^{**}	0.563
	Rosmarinus	0.626^{**}	0.533^{*}	-0.012
	Quercus	0.868^{**}	0.763^{**}	-0.271
	Cistus	0.895^{**}	n.c.[b]	n.c.[b]
	Bare soil	0.948^{*}	n.c.[b]	n.c.[b]

[a]Coefficient of partial correlations between GRSP and WR (log WDPT) controlling the effect of SOM.
[b]n.c.: not calculated because of the lack of SWR under this plant species and bare soil.
***,**,*; significant at $P \leq 0.001, 0.01, 0.05$ respectively.

In univariate ANOVA tests, differences in mycelium length were significant between groups ($P < 0.05$). Species with the most mycelium length were *Pinus* and *Quercus* and the less

Cistus (fig. 10). Pearson's correlation coefficients between mycelium length and WR, SOM content, pH and GRSP were not significant.

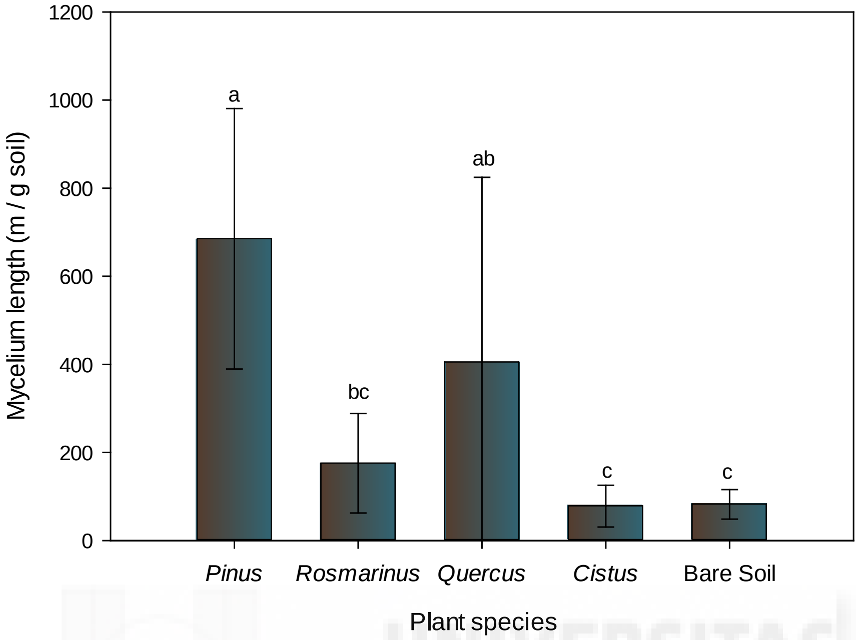

Figure 10: Mycellium length (m/ g soil) beneath the different species and bare soil (standard deviations in bars). Different letters show the statistically significant differences between the different species and bare soil with the Tukey test ($P \leq 0.05$).

Differences between species were also found for Ergosterol content. Mean content followed this order *Pinus* > *Quercus* > *Rosmarinus* > *Cistus* > bare soil (fig. 11).

Pearson's correlation coefficients with WR were significant (Table 4). In the case of *Pinus*, the relationship was especially strong (fig. 12) and did not disappear when we analyzed the partial correlation controlling SOM (Table 4). This is not the case with the rest of the species where the correlation between WR and Ergosterol, disappeared in the partial correlation controlling the effect of SOM (Table 4).

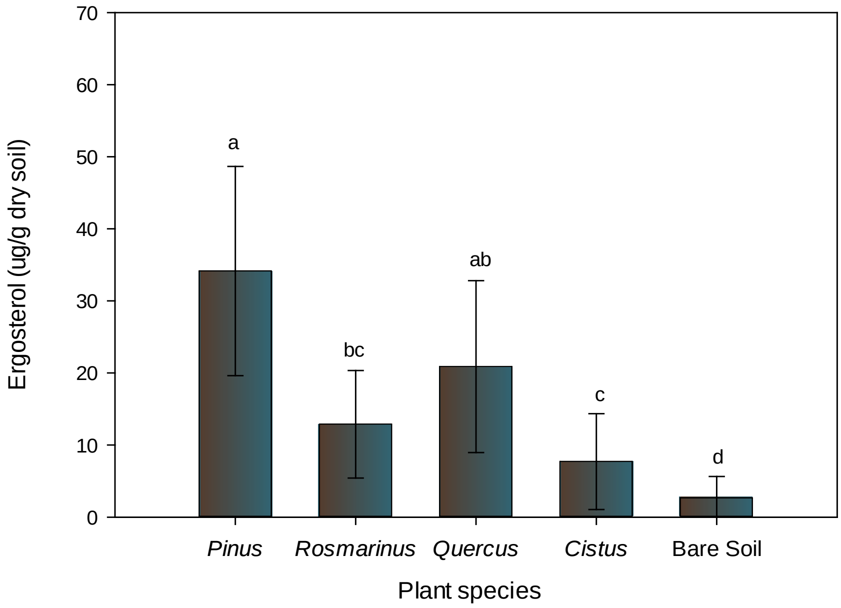

Figure 11: Ergosterol content beneath the different species and bare soil (standard deviations in bars). Different letters show the statistically significant differences between the different species and bare soil with the Tukey test ($P \leq 0.05$).

Table 4: Pearson's correlation coefficients between Ergosterol (log EG) and WR (log WDPT) for each species.

	Species	WR (log WDPT)	WR partial (SOM)[a]
	Pinus	0.905**	0.861**
	Rosmarinus	0.744**	0.243
Ergosterol (log EG)	*Quercus*	0.672*	0.331
	Cistus	n.c.[b]	n.c.[b]
	Bare soil	n.c.[b]	n.c.[b]

[a] Coefficient of partial correlations between Ergosterol and WR (log WDPT) controlling the effect of SOM.

[b] n.c.: not calculated because of the lack of SWR under this plant species and bare soil.

***,**,*; significant at $P \leq 0.001$, 0.01, 0.05 respectively.

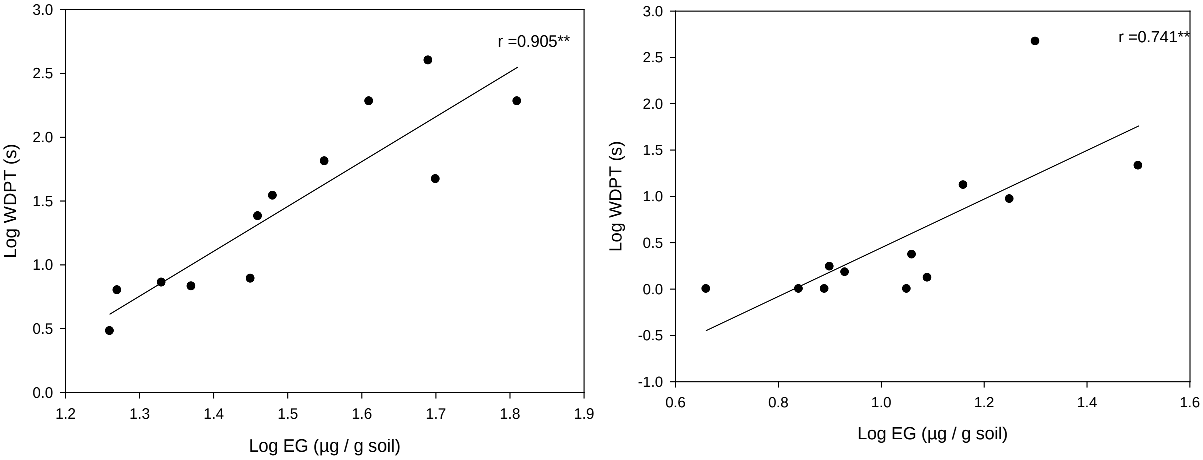

Figure 12: Relationship between WR (log WDPT) and Ergosterol (log EG) beneath Pinus (left) and Rosmarinus (right).

Results reveled a relationship with GRSP for *Quercus* (r= 0.681**) and particularly for *Rosmarinus* samples (r= 0.895**) (fig. 13).

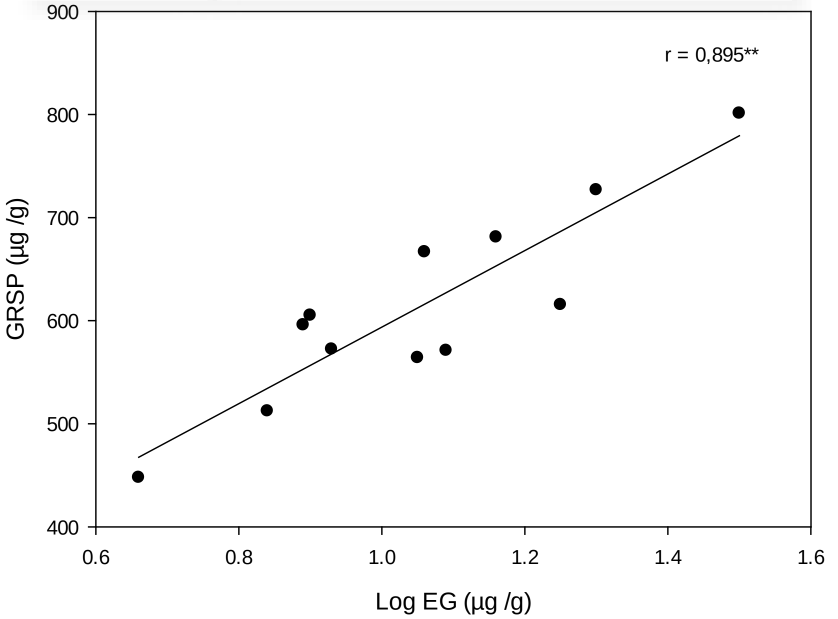

Figure 13: Relationship between GRSP and Ergosterol (log EG) beneath *Rosmarinus*.

DISCUSSION

Soil WR patchy distribution has already been reported in a large quantity of works carried out in forest soils in Mediterranean ecosystems (Martínez-Zavala and Jordán-López, 2009 and Mataix-Solera et al., 2007). In agreement with these researches, our results showed a high variability in persistence of WR, around 41% of samples were repellent, and the influence of SOM content on soil WR was evident (r=0.872 (fig. 3)). Persistence of WR was dependent on the influence of plant species. Depending on this factor, soil will be provided with a different input of organic compounds. In general, evergreen trees supply soil with considerably more organic material than shrubs, so as it was expected, we found the highest water repellent samples and total SOM content under the tree species studied (*Pinus* and *Quercus*). Severe soil WR can be induced by a high number of phenolic compounds found in the composition of leaves and plant tissues of oaks (Conde et al., 1998; Salminen et al., 2004) and resins, waxes, aromatic oils, and other substances in pines (Doerr et al., 1998).

Pinus is one of the genera that have received most attention for its influence in the development of SWR as much in Mediterranean ecosystems (Doerr et al., 2000) as in other ones (Buczko et al., 2002; Lichner et al., 2012). However, the influence of *Q. rotundifolia* in WR has still not been described, although there are studies in which soil WR is associated with different species of *Quercus* evergreen trees (Doerr, et al., 2000; Jordán et al., 2008) and shrubs (Arcenegui et al., 2008; Gimeno-García et al., 2011). A positive relationship between SOM content and WR has been reported several times (Mataix-Solera and Doerr, 2004; Mataix-Solera et al., 2007), especially if samples have been taken from the same soil type and beneath the same plant species. In other cases, poor or no significant correlations between SOM content and WR have also been reported (DeBano, 1981; Ritsema and Dekker, 1994; Scott, 2000). This inconsistency has been attributed to the fact that only a small amount of compounds of SOM are really implicated in WR (Mataix-Solera and Doerr, 2004; Zavala et al., 2009). WR might be controlled by the type and quality of organic matter rather than by its amount (DeBano et al., 1970; Wallis and Horne, 1992). In fact, we observed a different pattern of WR persistence between *Quercus* and *Pinus* despite having similar SOM content.

Lipid fraction has been commonly linked with hydrophobicity (DeBano, 2000). Our results of extractable lipid’s content would corroborate that hypothesis (fig. 5). Strong correlations between extractable lipid’s content and WR for *Quercus* and *Pinus* samples establish a clear relationship (fig. 5 and 6). Different concentrations in extractable lipid content could explain differences in WR persistence of samples from the same plant species. In agreement with us, De Blas et al. (2010) reported the effect of lipid humic fractions on SWR. They found the free lipids fraction to be the most relevant soil fraction inducing WR, although, they also outlined the importance of the particulate organic matter and extractable humic acids. Different patterns of persistence of SWR observed between *Quercus* and *Pinus* samples might be due to differences in composition of specific kind of lipids (fatty acids, sterols, waxes etc.) in their own organic materials. However, Horne and McIntosh (2000) did not find a clear correlation between WR with organic carbon content and with the quantity of lipid or any lipid fraction in New Zealand water repellent sandy soils. They suggested that the severity of repellency was not influenced by the total amount of lipids or any lipid fraction in the bulk soil but rather by orientation of amphipathic compounds. Samples for those studies were taken under very different plant covers; *Pinus* and *Eucalyptus* (De Blas et al., 2010) and different grass covers (Horne and McIntosh, 2000), so the origin and in turn the quality of SOM and its particular interaction with the chemical components of the surface could explain these differences. Thus, it would be interesting to know more about differences in extractable lipids and organic fractions between species to evaluate their possible specific influence on SWR. It could be also interesting to compare the interaction of these organic compounds with soils of different properties in their inorganic fraction (clay content, mineralogy, etc.).

The number of soil water repellent samples beneath *Rosmarinus* was significantly lower than *Pinus* and *Quercus*. Lower input of organic compounds to the soil would be the most consistent explanation. However, it is not so low, if we compare it with the results obtained by Gimeno-García et al. (2011) or Mataix-Solera et al. (2007), who found around 4% and 5% of water repellent samples respectively under this species. Mataix-Solera et al. (2007) carried out their study in the same area as us. The differences in severity of WR could be due to soil moisture, SOM content and its quality. In fact, if we compare both SOM content under *Rosmarinus*, we will observe that their data varied from 7.1 to 9.6, whilst our data goes from 7.9 to 13.86 (for wettable and water repellent samples, respectively). Our results would not be unusual if we took into account that *Rosmarinus* has a high relative wax input rate, which is based on leaf wax contents and characteristics of the ectorganic profile. That could be responsible for SWR (Mataix-Solera et al., 2007; Verheijen and Cammeraat, 2007).

On the other hand, organic material can influence pH, which could be strongly involved in the development of SWR. Our results showed the lowest pH in the most repellent samples, *Pine* and *Quercus*, which are the species with the highest SOM content. Organic materials would explain both differences in pH among species and the relationship between pH, SOM content and WR. In common with other authors (Martínez-Zavala and Jordán, 2009; Mataix-Solera et al.,

2007) we found a negative correlation between these parameters, i.e., repellency increases as pH reduces. Nevertheless, in agreement with these studies, the relationship seems to be influenced by SOM, as the partial correlations showed.

Traditionally, SWR has been associated with acidic soil, although it has already been reported in alkaline soils (Arcenegui et al., 2008; Mataix-Solera et al., 2007). Graber et al. (2009) concluded that SWR can develop in alkaline to neutral soils where Ca^{+2} ions are abundant, which are capable of interacting with fatty acids. That is in accordance with the model proposed by Diehl et al. (2010), in which the relationship between SWR and pH changes depends on an abundance of active protons (in our case it would be Ca^{+2}) and the organic matter functional groups, like fatty acids. The fatty acid structure is one of the most fundamental categories of biological lipids, and is commonly used as a building block of more structurally complex lipids. So, in accordance with that model, our results would not only explain the relationship between pH, SOM content and WR, but also in the case of *Pinus,* with lipids content too. In our case differences in organic functional groups could be an explanation for absence of correlation beneath *Quercus* with pH and the rest of the parameters.

According to other studies, SWR can however be caused by the activity of soil organisms. Studies found water repellency is closely associated with fungal growth and soil microorganisms (Jex et al., 1985; Rillig, 2005). Rillig et al. (2010) obtained a direct causal link between the growth of AMF mycelium and SWR under laboratory conditions. However, Hallett et al. (2009), under controlled conditions too, did not find a clear relationship. We also failed to find such a link at least in the first cm of soil depth. Our study measured mycelium length in soil, not only AMF mycelium, and the results suggest that not all fungal mycelium could be contributing in the same way to WR. Nevertheless, in general, our results seem to link stronger WR with fungal activity through the measurements of other related parameters (GRSP and ergosterol) instead of mycelium length. Young et al. (2012) obtained similar relationships for GRSP and ergosterol in two different ecosystems: grassland and an arable soil. On the other hand, under controlled laboratory conditions in which a specific AM fungi were introduced, Feeney et al. (2004) and Hallett (2009) did not find that link with glomalin and ergosterol respectively. The reason might lie in our analytical method (Bradford protein assay). With samples taken under field conditions, we could have measured protein compounds from a large diversity of AM fungi and maybe non-fungi derived compounds, as pointed out by Young et al. (2012); so that question remains still unclear.

In this study, we have tried to cover a large range of different fungal parameters in our analyses; AM fungi activity (GRSP), the total life fungal biomass (ergosterol) and mycelium length in soil. Every parameter offers us different information, so it is quite normal, especially in our case that weak correlations were found between mycelium length with GRSP and ergosterol. In the case of GRSP neither Borie et al. (2000) nor Rillig and Steimburg (2002) detected it. Production rates of glomalin are not always correlated with AM abundance (Treseder and Turner, 2007). Regarding ergosterol, normally active mycelium does not corresponded with mycelium length in soils.

Soil microbial biomass and its activity are influenced by the SOM content (Goberna et al., 2006). In fact, in our study, the link between fungal activity and SWR (at least under *Quercus* and *Rosmarinus*) appears to be influenced by them (Tables 3 and 4). Nevertheless, under *Pinus* we didn't find that correlation. We hypothesize that this relationship could be related more to the quality of SOM, in concrete with extractable lipids. *Pinus* obtained the biggest ergosterol content and mycelium length, this might be due to differences in SOM quality. Fungal:bacteria activity increases when soil C:N ratio increases (Kuijper et al., 2005). That suggests possible significant differences in C:N ratios between species. In Díaz-Pinés et al. (2011) C:N ratio obtained were higher under pine than under oak in a Mediterranean ecosystem. Those results support our results regarding the indirect relationship between fungal activity and WR as a consequence of the quality of SOM. *Pinus* and *Quercus* probably promote different microbial environments. How long lipids remain as free fractions will depend on their activity. Decomposition regimes could be also essential in the development and appearance of WR through the accumulation of polar substances (Franco et al., 2000).

On the other hand, the biggest glomalin content corresponded to samples of *Rosmarinus* (fig. 8). This result is logical as *Rosmarinus* is the only species in this study, which is associated particularly with AM fungi. These results might suggest a possible non-influence of GRSP in SWR, at least for the studied soil depth.

Nevertheless, it would be interesting to know more about differences in microbial environment beneath these species. More work would be required to characterize possible specific fungal and bacterial species responsible for inducing WR; in particular what specific compounds are really involved in the development of SWR. Our aim here was to study top- soil WR after the dry season, so maybe the fungal activity detected here, is not enough to be the main factor involved in the SWR at this soil depth (0-2.5cm). It could be also interesting to study their possible influence in SWR in other seasons and depths.

CONCLUSIONS

Water repellency is a complex phenomenon affected by a large quantity of soil parameters, which in turn are inter-connected. Thus, in our study we have tried to explain what are the main factors involved in its development in the top- soil layer. According to our results, in this environment and under these conditions, the quality of soil organic matter could be the major responsible factor. In general, the other parameters studied here seem to depend on it. In concrete, extractable lipids seem to be the principal factor.

Apart from *Pinus,* fungal activity appears to be related with SOM content. SWR found under *Pinus* seems to be the most influenced by fungi. Quality of SOM could be responsible for that. Presence or quantity of determined fungi or microorganisms might be related with the composition of SOM. Specific fungi through their role in the decomposition regimen could determine the persistence and permanence of SWR. Both the question of how long lipids remain as free fraction and accumulations of polar substances will depend on their activity.

Finally, if top-soil WR is caused by a fraction of SOM, the hypothesis of a possible ecological plant strategy makes it more acceptable. It would explain why WR appears in semiarid environments where improving water conservation is essential. Having patchy water repellent distribution in its area of influence could contribute to both channeling water deep into the soil profile to conserve it in depth near to their rhizosphere, and at the same time in reducing top-soil evaporation rates.

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CAPÍTULO 4

Relationships between soil water repellency and microbial community composition under different plant species in a Mediterranean semiarid forest

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ABSTRACT

Soil water repellency (SWR) can influence many hydrological soil properties, including, water infiltration, uneven moisture distribution or water retention. In the current study, we investigated how variable SWR persistence in the field is related to the soil microbial community under different plant species (*P. halepensis, Q. rotundifolia, C. albidus* and *R officinalis*) in a Mediterranean forest. The soil microbial community was determined through phospholipid fatty acids (PLFA). The relationships between microbiological community structure and the soil properties pH, Glomalin Related Soil Protein (GRSP) and soil organic matter (SOM) content were also studied. Different statistical analyses were used: Principal Component Analysis (PCA), ANOVA, Redundancy Analysis and Pearson correlations. The highest concentrations of PLFA were found in the most water repellent samples. PCA showed that microorganism composition was more dependent of the severity of SWR than the type of plant species. In the Redundancy Analysis, SWR was the only significant factor ($P < 0.05$) to explain PLFA distributions. The only PLFA biomarkers directly related to SWR were associated with Actinobacteria (10Me16:0, 10Me17:0 and 10Me18:0). All the results suggest that a strong dependence between SWR and microbial community composition.

Keywords: soil hydrophobicity; phospholipids fatty acids; microbial community structure; biohydrology; actinobacteria; Glomalin Related Soil Protein.

INTRODUCTION

Soil water repellency (SWR) can greatly influence the hydrology and the ecology of forest soils. The hydrological implications of SWR include reduced infiltration rates, enhanced runoff and overland flow that can exacerbate erosion and possibly flood risk (Cerdà et al., 1998; Coelho et al., 2005; DeBano et al., 2000, Doerr et al. 2000; Lichner et al., 2013; Moral García et al., 2005, Nicolau et al., 1996). SWR can also affect ecological processes including an allelopathic effect by suppressing the germination of competing vegetation (Stevens and Tang, 1985), and the improvement of water conservation into the soil profile by penetration to depth through preferential flow pathways (Moore and Blackwell, 1998; Robinson et al., 2010) and decreased evaporation rates through hydrophobic surface soils (Hallett, 2007). The origin of SWR has been researched in many studies, and although the role of soil microorganisms has been appreciated for many years (Bond and Harris, 1964), data on the interaction between SWR and soil microbial structure are scarce.

SWR, soil microbiology structure and activity are all functions of abiotic and biotic conditions; soil texture and mineralogy, soil moisture, climate, site specific conditions, chemical parameters, substrate availability, land use and plant communities (Gömöryová et al., 2013; Merilä et al., 2010; White et al. 2005; Zornoza et al., 2009). Previous research has shown that water repellent patches of soil differ from adjacent wettable soils in pH, moisture, SOM quality and quantity, and microbial biomass (Doerr et al., 2000), even beneath the same plant species (Lozano et al., 2013). When soil microorganisms decompose SOM, hydrophilic compounds are more resistant to be degraded, potentially resulting in an accumulation of hydrophobic compounds over time. The capacity of soil microorganisms to degrade different SOM compounds depends upon species composition, so this may affect changes in SWR at the microsite scale (such as the presence of soil water repellent patches; Mülleret al., 2010). Early studies suggest that SWR might be caused by substances produced by the activity of certain fungi species (Savage et al., 1969). Based on these assumptions, the microbiology structure in soil samples with different SWR persistence and under different plant species was studied.

This contribution is a continuation of previous research (Lozano et al., 2013), where we concluded that the quality of SOM, in specific a lipid fraction, could be the main factor involved in SWR. However, in the case of forested land with *Pinus*, it was also postulated that soil microbiology could be another major factor, but this was not investigated. In the current study, microbial community structure measured with phospholipid fatty acid (PLFA) analysis, was compared to the persistence of SWR. The sites studied are the same as Lozano et al. (2013). The area has a Mediterranean climate and sampling was carried out under trees (*Pinus* or *Quercus*) and shrubs. The sites were sampled at different locations to give a range of SWR severity that could then be measured for microbial community structure. Studying soil microbiology structure could help to understand the possible influence of SWR in soil microbiology, or in contrast, the possible influence of soil microbiology in the development of SWR. Understanding of the relationship is of great ecological importance, especially given that *Pinus sp.* has been closely related to SWR (Lozano et al., 2013; Mataix Solera et al., 2007) and is commonly used in afforestation projects.

MATERIAL AND METHODS

Study area

The study area is located in the 'Sierra de la Taja' (38°23'N; 0°59'W) near Pinoso, in the province of Alicante (SE Spain). The region has a semi-arid Mediterranean climate with a mean annual precipitation of 277.5 mm and a mean annual temperature of 15.8°C ranging from 7.8°C in January to 24.1°C in August (average 1980-2010). The area of the 'Sierra de la Taja' is approximately 500 ha. Soil samples were taken under similar conditions with respect to soil type, geology, plant distribution and slope. The soil is a Lithic Xerorthent (Soil Survey Staff, 2014) with a calcareous nature developed over Jurassic limestone. The soil texture in the area is loam, with 36% sand, 49% silt and 15% clay.

Three types of vegetation covered the area: (i) *Pinus halepensis* Miller (approximately 40 years old), (ii) *Quercus rotundifonlia* and (iii) shrub perennial vegetation comprised mainly *Quercus coccifera* L., *Rosmarinus officinalis* L., *Juniperus oxycedrus* L., *Cistus albidus* L., *Brachypodium retusum* Pers. (Beauv.), *Stipa tenaccissima* L. and *Pistacia lentiscus* L. Tree and shrub species are mixed in the study area, but as a consequence of the relatively low density of vegetation, it was possible to carry out the sampling avoiding interference between the different species.

Soil sampling

Samples were taken in September 2011, when the SWR is expected to be at its peak after the typical Mediterranean summer drought (Doerr et al., 2000). Soils were sampled from the first 2.5 cm of the A horizon at microsites (approximately 100 cm^2) beneath each of the four most representative species (*Pinus halepensis, Rosmarinus officinalis, Quercus rotundifolia* and *Cistus albidus*; n=15 per species). Both plants and microsites under each plant were randomly selected within a 100 m x 100 m area. Based on water repellency results, we selected three soil samples per species for PLFAs analysis, including the variability of SWR classes found in some cases per species when possible (e.g. *Rosmarinus* and *Quercus*). We analysed the relationships between PLFAs with soil properties related with SWR such as: soil organic matter (SOM) content (Martínez-Zavala and Jordán López, 2009), glomalin related soil protein (Rillig, 2005) and pH (Lozano et al., 2013; Mataix-Solera et al., 2007).

Laboratory methods

Soil samples were dried at room temperature (20-25°C) to a constant weight and passed through a 2 mm sieve to remove coarse soil particles before soil analysis. Soil pH was measured in aqueous soil extract in de-ionised water (1:2.5 w:s) at 25°C. SOM content was analysed by rapid dichromate oxidation of organic carbon (Nelson and Sommers, 1996). The persistence of SWR was measured by the Water Drop Penetration Time (WDPT) test (King, 1981). The logarithm of the WDPT value in seconds was used and samples were taken as water repellent if the value of log (WDPT) was > 0.7. Based on the SWR of the 12 samples analysed, they were divided in three groups; strong (log (WDPT) > 1), slight (log (WDPT) > 0.7 and < 0.9) and wettable (log (WDPT) < 0.7) (Table 1).

Glomalin Related Soil Protein (GRSP) is a glycoprotein produced primarily by arbuscular mycorrhizal fungi (AMF) and is contained within their hyphae walls (Wright and Upadhyaya, 1996). When the AM hyphae die and decompose, they are thought to leave a glomalin residue in the soil (Treseder and Turner, 2007). GRSP was measured as the Easily Extractable Glomalin, which corresponded to the fraction of protein most recently deposited into the soil. GRSP was extracted from 0.25 g subsamples of soil with 2 ml citric acid buffer, pH 7.0 at 121°C for 30 min. Protein in the supernatant was determined by a Bradford assay (Wright and Upadhyaya, 1996).

Table 1: Soil properties of the different samples used in the study.

Soil sample	WDPT (s)	Log WDPT (s)	WR class	pH	SOM (%)	GRSP
Quercus 1	498	2.70	Strong (++)	7.8	17.0	43.4
Quercus 2	1623	3.21	Strong (++)	8.1	16.9	41.1
Quercus 3	5.1	0.73	Slight (+)	8.0	13.4	42.4
Pinus 1	7	0.85	Slight (+)	7.9	12.4	35.3
Pinus 2	6.8	0.83	Slight (+)	7.9	11.3	32.0
Pinus 3	7.3	0.86	Slight (+)	8.0	11.5	37.1
Cistus 1	1	0.10	Wettable (-)	8.2	5.3	27.3
Cistus 2	1	0.10	Wettable (-)	8.1	7.1	28.6
Cistus 3	1	0.10	Wettable (-)	8.1	5.9	24.9
Rosmarinus 1	1	0.10	Wettable (-)	8.0	6.5	32.2
Rosmarinus 2	21.25	1.33	Strong (++)	7.9	15.2	50.1
Rosmarinus 3	13.25	1.12	Strong (++)	8.0	13.3	42.7

WDPT: Water Drop Penetration Time; WR: Water Repellency; SOM: Soil Organic Matter content; GRSP; Glomalin Related Soil Protein

Phospholipid fatty acids (PLFA) analysis was carried out as described in Bossio et al. (1998). Briefly, fatty acids were extracted from 8 g soil using chloroform: methanol: phosphate buffer. They were then separated from neutral and glycolipid fatty acids on a solid phase extraction column (0.58 Si; Supelco Inc., Bellafonte, PA, USA). After mild alkaline methanolysis, samples were analyzed using a Hewlett Packard 6890 Gas Chromatograph with 25 m Ultra 2 (5% phenyl)-methylpolysiloxane column (J and W Scientific, Folsom, CA, USA). Fatty acids were quantified by comparison of the peak areas with those of an internal standard 19:0 peak. The peaks were identified using bacterial standards and identification software from the Microbial Identification System (Microbial ID, Inc., Newark, DE, USA), with 48 fatty acids identified.

Fatty acids nomenclature used was that described by Frostegåard et al. (1993). The fatty acids i15:0, 15:0, a15:0, i16:0, 16:1ω7, i17:0, a17:0, cy17:0, 17:0, 18:1ω7 and cy19:0 were chosen to represent bacteria (Frostegård et al., 1993). The unsaturated PLFA 18:2ω6 was used as an indicator of fungal biomass (Federle, 1986). PLFAs cy17:0, 18:1ω7c, cy19:0, 17:1ω9c, 16:1ω9c, 18:1ω9c and 15:1ω4c were chosen to represent Gram-negative [G−] bacteria (Zelles et al., 1994). The branched, saturated i14:0, i15:0, a15:0, i16:0, i17:0 and a17:0 were chosen to represent Gram-positive [G+] bacteria (Zelles et al., 1994). The PLFA 10Me16:0, 10Me17:0 and 10Me18:0 were selected as indicators of actinobacteria (Zelles et al., 1994). The PLFA 16:1ω5 was used as indicator of vesicular–arbuscular mycorrhizal (VAM) fungi (Olsson et al., 1995) but has also been found in bacteria (Nichols et al., 1986). The total biomass was estimated as the sum of all the extracted PLFA (total PLFA). The ratio PLFA / SOM was calculated.

Statistical analyses

Principal Components Analysis (PCA) was carried out using MVSP 3.2 (Multivariate Statistical Package, Kovach Computing) to analyze the importance of different PLFA for different species and soil WR class. Normality and homogeneity of variances for all data were tested, and log transformation was made for WDPT results. In addition, a Redundancy analysis (RDA) was used to explore the relationship between the microbial community composition and soil characteristics. Samples with similar PLFA profiles have similar scores and will therefore group closer together when plotted. Soil properties were tested for significant contributions to the variation in the PLFA data using the Monte Carlo permutation test ($P < 0.05$). Soil properties are represented by vectors distributed on 2 axis. The correlation with the axis is measured by both angle with the axis and magnitude of the vectors. Small angle and great magnitude means a great correlation. RDA can be influenced by rare fatty acids. Fatty acids that only appear in a few samples are usually unreliably represented, as their values are near the detection limit. Hence, fatty acids that were present in less than 25% of the samples were omitted to avoid this problem in developing the analysis. RDA was performed using CANOCO for Windows, Version 4.54.

ANOVA-one way was also used to evaluate the differences in the ratio PLFA nmol/g soil and SOM content (g 100 g^{-1} soil) between the three SWR classes. Pearson's correlation coefficients (r) were calculated to quantify the linear relationship between soil parameters and PLFA biomarkers. Statistical analyses were performed using the SPSS 11.5 package (© SPSS Inc, 1989).

RESULTS

Microbial community structure

PLFA data from the soil under the four plant species where subjected to PCA. Fig. 1 (a and b) shows the distribution of PC scores on the first two axes. PC1 explained 39% of the variance in PLFA composition, while PC2 explained a further 20%. To determine whether plant species or SWR explains the results, PCA scores were represented in both plant species and SWR classes (fig. 1(a) and (b)). Variations in PLFA composition were clearly explained when samples were separated by WR class (fig. 1(a) and (b)). Axis 1 separated almost all the samples into water repellent and wettable, while axis 2 separated strong from slight water repellent and wettable samples. However, the separation between species was just clear for *Pinus* and *Cistus* in Axis 1.

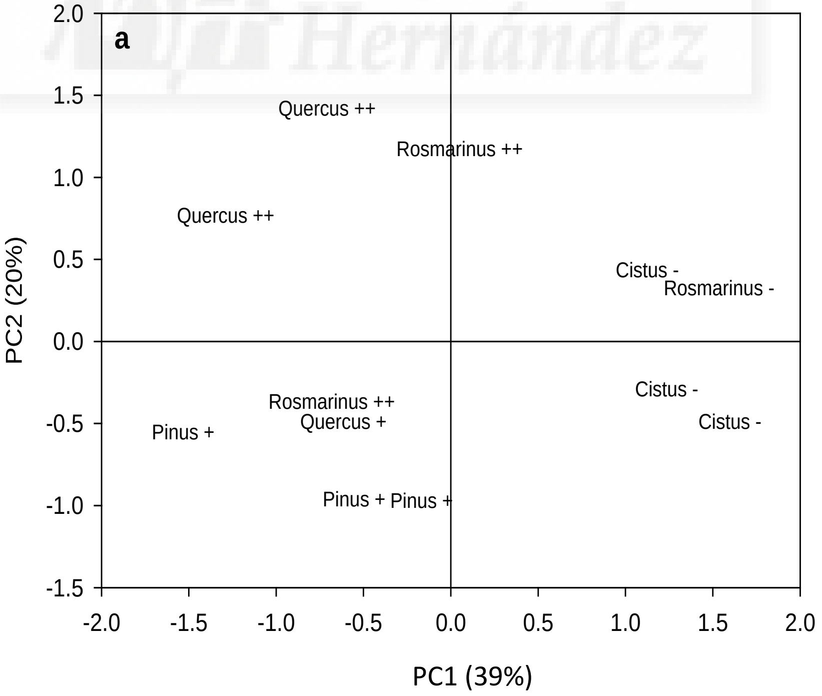

Figure 1 a): Scores of the first two principal components (PCs) analyses of the phospholipid fatty acids (PLFAs). Distribution of the scores of the different species and their WR class. The different symbols indicate the WR classes; (-) = wettable, (+) = slight and (++) = strong WR.

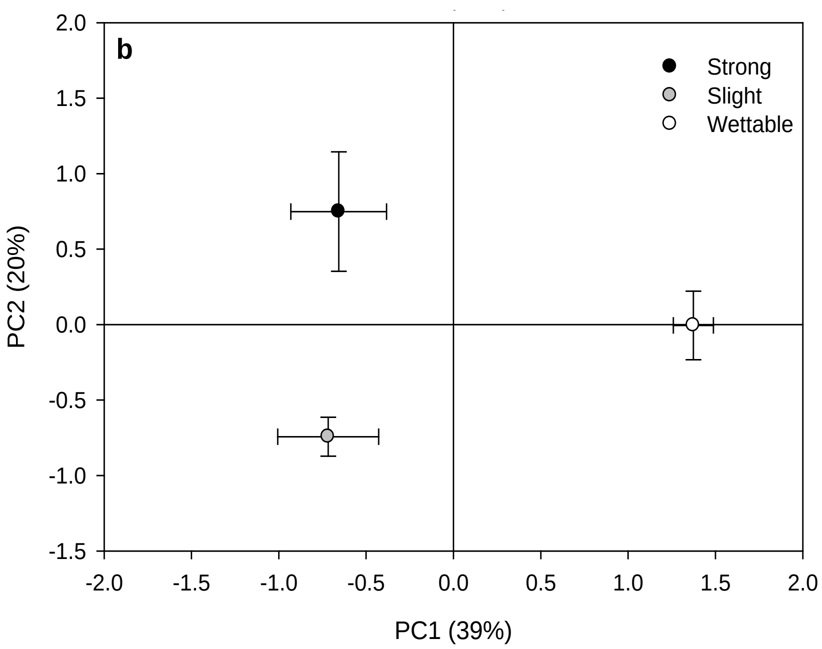

Figure 1 b): Scores of the first two principal components (PCs) analyses of the phospholipid fatty acids (PLFAs). Distribution of samples according to the WR class; values represented correspond with the means and standard errors of the scores of each class.

The RDA performed on all data (fig. 2 and 3) showed that the first two axes explained 88.3% of the total variation. Axis 1 separated WR samples from wettable samples and explained 32.1% of the variation, whilst Axis 2 explaned 56.2% of the variation.

The variables GRSP, SWR and SOM accounted for a large amount of the variation in the distribution along Axis 1 and thus with samples that showed water repellency. However, only the SWR variable was significant ($P \leq 0.05$) in explaining the PLFA data. In contrast pH was not associated with these soils (fig. 2). Vectors defining the axis are strongly related with the PLFA that appears in that axis (Ter Braak, 1987).

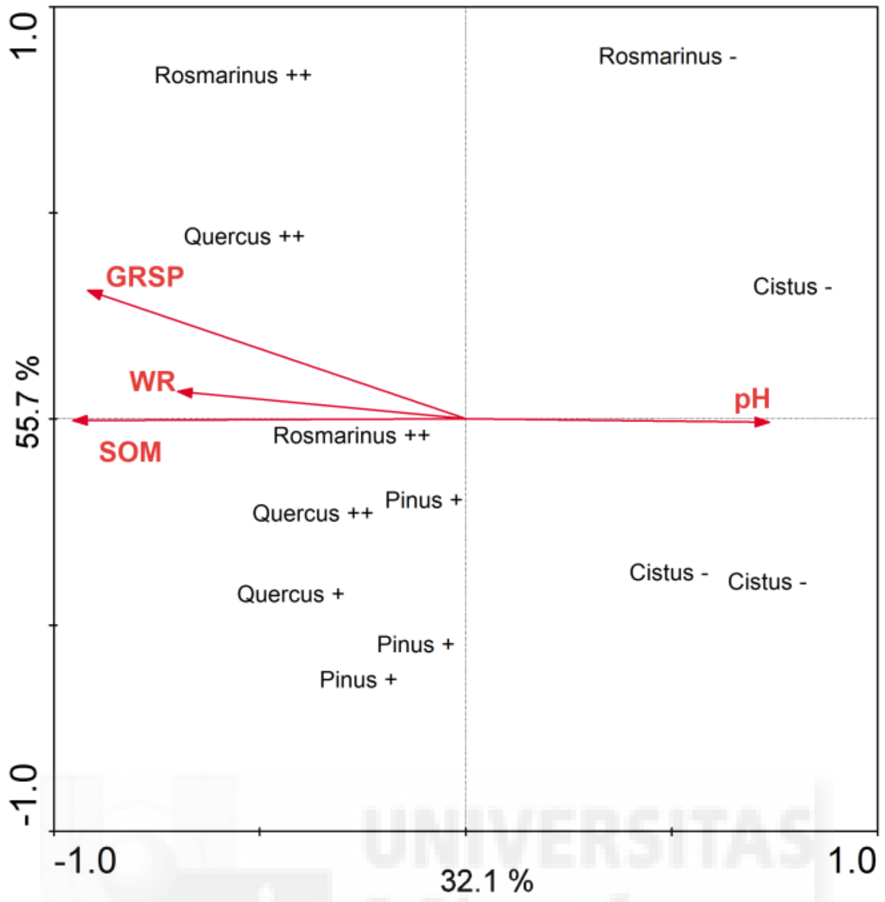

Figure 2: Samples and soil characteristics biplots from RDA performed on the relative concentration of PLFAs in soils under the different species studied: *Quercus*, *Pinus*, *Cistus* and *Rosmarinus*. SOM: soil organic matter; WR: water repellepency; GRSP: glomalin-related soil protein.

Certain PLFA were strongly associated with water repellent samples, which were characterized by high concentrations of the saturated PLFA 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 18:02OH, the unsaturated PLFA i14:0, 16:1w7t and cy17:0 that are mainly representative of bacteria (fig. 3). The PLFA 18:2w6, representative of fungi, was also associated with these samples. These samples were clustered with metilated PLFAs 10Me17:0 and 10Me18:0 which are indicators of actinobacteria.

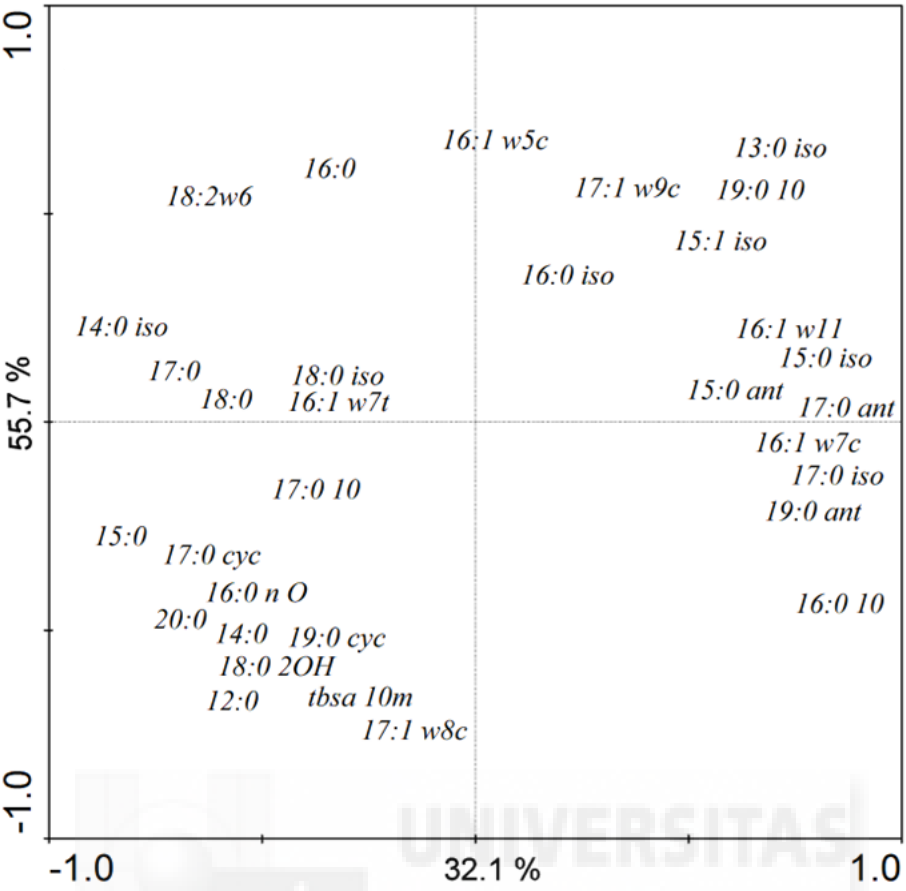

Figure 3: Loadings plots from RDA performed on the relative concentration of PLFAs in soils under the different species studied: *Quercus*, *Pinus*, *Cistus* and *Rosmarinus*. SOM: soil organic matter; WR: water repellepency; GRSP: glomalin-related soil protein.

Total PLFA content

Significant differences in total PLFA content were found between water repellent and wettable samples. The highest concentrations were found in water repellent soil samples (fig. 4). Nevertheless, the higher content of microbial PLFA per g of SOM content was found in wettable samples. The contents of microbial PLFA in strongly and slightly water repellent soil samples were similar (fig. 5).

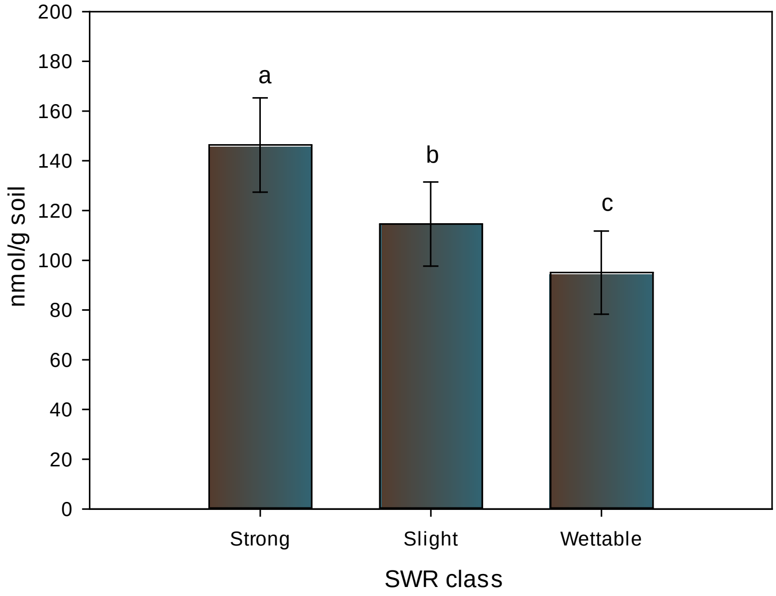

Figure 4: Total PLFAs content (nmol/g soil) in samples grouped by the different soil WR class (standard errors in bars). Different letters show the statistically statistically significant differences between the different soil WR classes determined with the Tukey test (P ≤ 0.05).

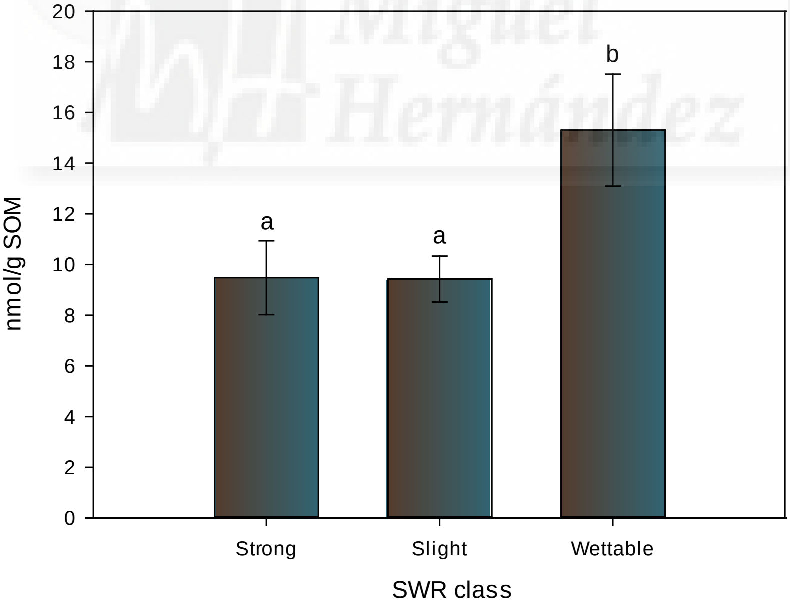

Figure 5: Total PLFAs content (nmol/g SOM) for samples grouped by the different soil WR class (standard errors in bars). Different letters show the statistically statistically significant differences between the different soil WR classes determined with the Tukey test (P ≤ 0.05).

Biomarkers and its relationship with environmental parameters

Significant correlations were found between physico-chemical parameters and biomarkers (Table 2). WR was correlated with actinobacteria and total PLFAs. SOM content was correlated with all the parameters with the exception of Gram-positive bacteria. However, all the parameters were strongly correlated with GRSP. No correlations were found with pH.

Table 2: Pearson's correlation coefficients of PLFAs biomarkers with other soil parameters.

	Bact/Fung	**Gram +**	**Gram -**	**Actinobacteria**	**Fungi**	**Total**
Log (WDPT)	0.359	0.573	0.523	**0.676***	0.451	**0.608***
SOM	**0.811****	0.526	**0.732****	**0.758****	**0.638***	**0.736****
GRSP	**0.913****	**0.670***	**0.896****	**0.623***	**0.797****	**0.782****
pH	-0.497	-0.516	-0.389	-0.514	-0.524	-0.507

WDPT: Water Drop Penetration Time; WR: Water Repellency; SOM: Soil Organic Matter content; GRSP; Glomalin Related Soil Protein; ***, **, *; significant differences at P ≤ 0.001, 0.01 and 0.05 respectively.

DISCUSSION

The results of this study indicate that, in the calcareous soil in semiarid conditions that was studied, patterns in soil microbial community structure were clearly grouped better by SWR class than by plant species. Soil water repellent samples were more similar in microorganism composition between themselves than from samples within the same plant species (fig. 1). This is not unusual in the Mediterranean region, where vegetation patterns exhibit a high spatial heterogeneity (Valladares et al., 2002) comprising a mixture of trees and shrubs that may interact. However, the in the patchy distribution of soil WR seems to influence the microbial community structure, including the prevalence of specific groups of microorganisms like actinobacteria, more than vegetation in our study. This finding is supported by Brockett et al. (2012), who found soil moisture and SOM to be more important than vegetation for soil microbial community than broad biogeographical regions. Their study was carried out in a Canadian forest, where climate and possibly the prevalence of SWR would be very different from the Mediterranean site that we studied. The interaction between SWR and soil moisture is complex (Doerr et al., 2000), and to our knowledge the interplay between these properties and the development of soil microbial structure has not been examined to date. There is a challenge to understand if microbial community structure is driven by or drives SWR. As biological impacts on SWR occur at the microscale (Rillig et al., 2005; Roper, 2004), small-scale measurements could disentangle spatial variation across millimeter resolution that may drive different microbial communities over small distances.

Many studies have found plant species composition to be a good indicator of belowground community composition (Mitchell et al., 2010; Thoms et al., 2010). In these studies the differences between plants of the same species cannot be distinguished, replicates of samples were combined for microbiological analysis, so differences found represent the general influence of tree species in a large area. However, our results are not in complete disagreement as SWR persistence is closely related to SOM content and quality, which in turn is dependent on vegetation cover (Lozano et al., 2013; Mataix-Solera et al., 2007). The influence of the dominant plant species on the input of SOM to soils is mainly attributed to the different amount and chemical composition of litter and root exudates (Graystone et al., 1996; Zak et al., 2003). Nevertheless, SWR has a patchy distribution even under the same vegetation cover type (Martínez-Zavala and Jordán-López, 2009), and this seems to influence soil microbial structure. Hydrophobic compounds of SOM may derive directly from the decomposition of plant leaves that contain considerable amounts of waxes, aromatic oils, resins and other hydrophobic substances (Doerr et al., 2000), which are generally more resistant to microbial degradation than hydrophilic ones. These hydrophobic compounds may select for microorganisms capable of producing enzymes to utilize them in soil.

Our results agree with the hypothesis about SWR as an obesity syndrome proposed by Müller and Deurer (2011). The appearance of hydrophobicity in the soil is due to the accumulation of hydrophobic substances in soil at a higher rate than its capacity of degradation. The smallest microorganism / SOM content ratio was found under the water repellent samples (fig. 3(b)). These results might indicate that the input of organic matter is impairing the input of microorganism biomass (SOM content increases faster than the microorganisms), which implies disequilibrium in the mineralization rates. This may produce shifts in the microbial community. Many studies point out the important role of biotic factors (microbial community structure and activity) in SOM mineralization, in addition to the environmental conditions and organic matter content (Blagodatsky et al., 2010; Garcia-Pausas and Paterson, 2011).

The persistence of SWR depends, in part, on the presence of microorganisms capable of degrading water repellent compounds. In our soil samples, actinobacteria seemed to be the only group directly correlated to SWR (Table 2). The results obtained in the study of Roper (2004), in which wax degrading bacteria were isolated and identified from water repellent soils, showed that a significant proportion of the wax-degrading bacteria belonged to actinobacteria. Many of these microorganisms have the potential to degrade waxes that cause SWR through the production of surface active molecules that facilitate their degradation (Roper, 2004). However, many PLFA mainly from bacteria were clustered with water repellent samples (fig. 2(b)). These PLFA might have been present in a higher concentration in specific bacteria, which may have been important in the degradation of water repellent compounds. These bacteria may correspond to another bacteria isolated by Roper (2004), which did not correspond to actinobacteria.

No significant correlation has been found between the pH and the PLFA biomarkers in our results. This is different to a number of studies that identified soil pH as one of the main environmental factors driving soil microorganisms functions and structure (Bääth and Anderson 2003; Hackl et al., 2005; Högberg et al., 2007). However, the range of the pH values in our soils is very low (7.8 – 8.2) which is probably the main reason for the lack of the significant correlations.

The development of SWR from the metabolic products of micoorganism activity was first demonstrated decades ago (Bond and Harris, 1964) and SWR development has also been directly related to certain fungi species (Rillig et al., 2005; Savage et al., 1964; White et al., 2000). In our research, we found a relationship between field SWR and microbial community structure, particularly actinobacteria. The underlying mechanisms driving this relationship needs further study as it could not be concluded whether SWR was a product of the microbial community or vice versa.

CONCLUSIONS

In the Mediterranean forest context, SWR was found to be related to microbial community composition. The accumulation of different hydrophobic compounds might be causing the shifts in microbial community structure or the soil microbial structure could be conditioned by the presence of SWR. Differences in the soil parameters (moisture, temperature, GRSP accumulated, quality of SOM) at the spatial microscale might be also responsible for the shifts in the microbial community composition, due to their role in the SOM mineralization. Our results suggest that actinobacteria are predominant in SWR samples, so further investigation of their impact on SWR development or competitive ability in water repellent soils might unravel underlying processes. We provided further evidence that soil hydrology and microbial communities are closely linked properties in soils.

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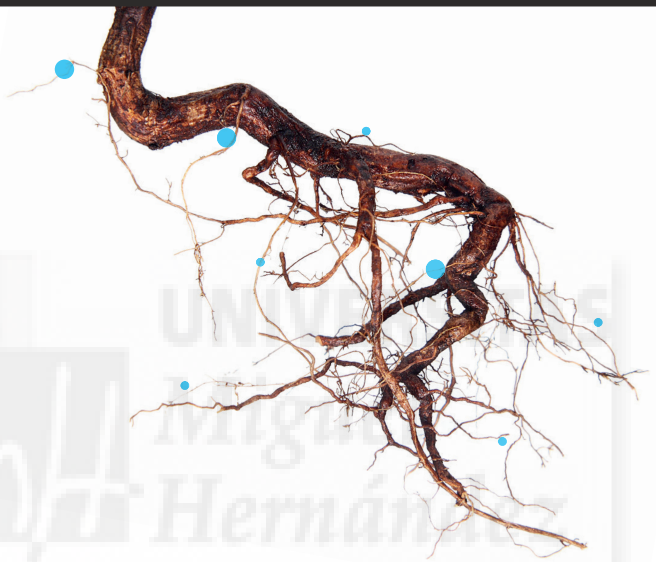
CONCLUSIONES / CONCLUSIONS



CONCLUSIONES

Esta investigación aporta conocimientos acerca del comportamiento de la glomalina del suelo a las temperaturas que se pueden alcanzar durante un incendio forestal, así como de su respuesta inmediata y a medio plazo tras la perturbación. A su vez, también contribuye a un mejor conocimiento sobre los factores involucrados en el desarrollo de la repelencia al agua en condiciones naturales y sus consecuencias sobre la comunidad microbiológica del suelo. Los resultados obtenidos nos han permitido obtener las siguientes conclusiones:

i. La glomalina es sensible a las temperaturas alcanzadas en el suelo. La respuesta de la GRSP a la temperatura varía dependiendo del tipo de suelo (y en especial de su textura), así como otras propiedades del mismo, entre las que destaca la agregación y el contenido de materia orgánica. La respuesta del contenido de materia orgánica al fuego es muy similar entre suelos, mientras que la de la repelencia al agua no siempre es predecible. El estudio combinado de diferentes parámetros del suelo sensibles a las temperaturas, podría proporcionar valiosa información acerca de la severidad del fuego en el suelo.

ii. El fuego provoca efectos en el contenido de la glomalina del suelo tanto a corto, como a medio plazo. A corto plazo, variaciones significativas en el contenido de GRSP indican mayor severidad, no teniendo porqué observarse dichos cambios en incendios de baja severidad. Por el contrario, la ausencia de variaciones en el contenido de GRSP a medio plazo y durante diferentes épocas del año, es indicativo de los efectos del fuego en el tiempo sobre GRSP y por ende, sobre las comunidades microbiológicas y vegetales. Estos efectos en la evolución de GRSP se observan incluso en incendios de severidad no muy alta, lo que enfatiza la necesidad de monitorizar las zonas afectadas por incendios. El tipo de cubierta vegetal, sin embargo, no se ha mostrado como un factor influyente sobre la evolución de los stocks de glomalina en el tiempo tras el fuego.

iii. La presencia de la repelencia al agua en suelos forestales calcáreos es un hecho, así como su variabilidad espacial. Es una propiedad muy compleja y son muchos los factores implicados en su presencia e intensidad, pero sin duda es la especie vegetal en última instancia, la mayor involucrada a través de la materia orgánica que aporta al suelo. En este sentido, el factor más determinante en la persistencia de los parches de repelencia que aparecen en el suelo no sólo es la cantidad de materia orgánica en el suelo, sino la calidad de la misma, recayendo el mayor peso sobre el contenido de lípidos. Del tipo y cantidad de materia orgánica también va a depender la influencia de los microorganismos en la misma, como lo demuestran los resultados de GRSP y ergosterol. Estos parámetros aparecieron generalmente correlacionados con la repelencia al agua de manera indirecta a través de la materia orgánica.

iv. La hidrología del suelo y las comunidades microbianas son propiedades muy relacionadas en los suelos. Las diferentes condiciones (humedad, materia orgánica, biomasa, etc.) que se dan entre los parches hidrofóbicos e hidrofílicos en el suelo son claramente condicionantes de la estructura de la comunidad microbiana y viceversa, ya que de ellos también depende la degradación de la materia orgánica y sus transformaciones. Hemos comprobado, que la similitud de la estructura de las comunidades microbianas depende en mayor grado de la severidad de la repelencia al agua que de la especie vegetal. Los microorganismos más relacionados con la presencia de la repelencia son las actinobacterias, microorganismos generalmente asociados a la degradación de compuestos más recalcitrantes, como son los compuestos hidrofóbicos.

CONCLUSIONS

This research contributes to the understanding of glomalin-related soil protein response to wildfire disturbance. In concrete, it provides new knowledge about GRSP response to heating temperatures and to wildfire impact in the short and medium term. At the same time, it contributes to a better understanding of the main factors involved in soil water repellency development in forests and its consequences on the soil microbial community. The results previously discussed allow the obtaining of the following conclusions:

i. Glomalin is sensitive to temperatures reached in soil during a wildfire. GRSP response varies depending on soil texture. Other soil properties like aggregates and soil organic matter content also influence the GRSP response to temperature. Organic carbon content response to fire is similar between soil types, whilst soil water repellency is not always predictable. The study of GRSP, together with other sensitive soil parameters to temperatures can provide useful information about fire severity in soils.

ii. Fire alters GRSP stocks in short and medium term. Immediately after fire, significant variations are indicative of higher fire severity. Fires with a low severity do not necessarily alter GRSP stock. On the contrary, no changes in the medium term and across the different seasons over a year in GRSP are indicative of fire impacts over time on microbiological and plant communities. These effects appear even in fires with a low severity, which emphasizes the importance of monitoring changes over time after wildfires. The type of plant cover however does not seem to have an influence on GRSP stocks over time.

iii. Soil water repellency presence in calcareous forest soils is a fact, and is very variable. It is a complex property and there are many factors involved in its presence and intensity. However, the most important factor is the vegetal specie through the organic material input to the soil. In this sense, not only the quantity of the organic material is the dominant factor influencing the persistence of water repellent patches on soil, but also its quality. In concrete, the extractable lipids seem to be the main factor involved. At the same time, quality of organic matter influences the microbial community.

iv. Soil hydrology and microbial communities are properties well correlated in soils. Differences in soil parameters (moisture, organic material, biomass etc.) between wettable and water repellent samples clearly influence soil microbial structure and *vice versa*. Soil microbial community structure is more influenced by the patchy distribution and the persistence of soil water repellency than the plant species. Some species, like actinobacterias, are predominant in soil water repellent patches.

TESIS DOCTORAL

Elena Lozano Guardiola

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Sensitivity of glomalin to fire effects on soil and its relationship with water repellency in Mediterranean forests soils

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