

# GUÍA DE CAMPO SERELLES. PARQUE NATURAL DE LA SIERRA DE MARIOLA, ALCOI. ALICANTE

SALIDA DE CAMPO DEL IX SIMPOSIO NACIONAL SOBRE CONTROL DE LA DEGRADACIÓN Y RECUPERACIÓN DE SUELOS. 28 MAYO 2020

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## Guía de Campo

## Serelles. Parque Natural de la Sierra de Mariola, Alcoi, Alicante

28 mayo 2020

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### Introducción

La excursión forestal del congreso consiste en una ruta de senderismo desde Alcoi por la Sierra de Mariola, concretamente a la zona de Serelles (Figura 1). Durante la excursión se visitarán zonas forestales afectadas por incendios y que han sido sometidas a diferentes manejos post-incendio, zonas no quemadas con y sin tratamientos silvícolas y se discutirán resultados de diferentes estudios realizados en la zona. En el trayecto se realizarán también paradas para describir varios perfiles de suelos forestales típicos de la región. La comida será un picnic en un área recreativa a cargo del Ayuntamiento de Alcoi. Por la tarde se prevé una visita corta al Parque Natural de la Font Roja.



Figura 1. Recorrido de la excursión

Datos técnicos: distancia total entre ida y vuelta: 12 km aprox.; desnivel: comenzamos en 618 y la última parada está a 1012 m s.n.m.

Descarga la ruta en Wikiloc: https://es.wikiloc.com/rutas-outdoor/sierra-de-mariola-condegres-2020-46051036

## Clima y relieve

La zona se encuentra bajo un clima típico Mediterráneo. La precipitación media anual es de 490 mm, con los máximos en octubre-noviembre (71 mm). La temperatura media anual es de 14,8 °C (verano: 22,7 °C, invierno: 7,9 °C).

El relieve es abrupto y en su mayor parte conserva las terrazas de antiguos cultivos en los que tras el abandono de la actividad agrícola hace décadas se ha ido recolonizando por masa forestal. El Parque Natural de la Sierra de Mariola tiene una extensión de 17257 ha y se localiza entre las comarcas de la Hoya de Alcoi, Condado de Cocentaina y el Valle de Albaida con su cota máxima de 1390 m s.n.m., correspondiendo a la cima del Montcabrer. La zona a visitar, Serelles se encuentra en la parte baja más cercana a Alcoi.

#### Geología

Este recorrido se localiza próximo al contacto entre el Prebético externo e interno, lo que confiere una cierta complejidad estructural a la zona. Tectónicamente la Sierra de Mariola presenta una dirección estructural N45E algo diferente a la que preferentemente suelen presentar las estructuras béticas (pliegues y cabalgamientos) que es N70E. Se trata de una

antiforma (pliegue en champiñón) limitada por depresiones (Cuenca de Alcoy y Sinclinal de Agres) y elevaciones como el diapiro de Castalla (DPA-IGME, 2007).

Si bien las series mesozoica y el paleógena se pueden establecer con cierta precisión, no ocurre lo mismo con el relleno mioceno de la Cuenca de Alcoy que presenta importantes cambios laterales de facies, por lo que es difícil correlacionarlo de unos puntos a otros, dificultando su estudio. El itinerario que aquí se presenta discurre prácticamente en su totalidad por estas formaciones, por lo que en ocasiones puede resultar difícil situarse con detalle en la posición estratigráfica precisa.

En el núcleo central de la Sierra de Mariola predominan los materiales calcáreos cretácicos que conforman las zonas elevadas de este relieve. En las vertientes suroccidentales de la sierra afloran preferentemente calizas y dolomías eocenas, que dan paso a materiales mayoritariamente margosos de edad Mioceno (facies Tap) hacia la zona de trabajo. La deformación sufrida por estos materiales queda reflejada en la estructura anticlinal de Mariola, la cual sufre una brusca terminación por el Oeste hacia la depresión de Cocentaina-Muro de Alcoy. La compresión que sufrieron los materiales durante el plegamiento favoreció la extrusión de las arcillas y yesos del Triásico Keuper aprovechando las fallas asociadas a esta tectónica (IGME, 1995). Estos materiales evaporíticos no se observan a lo largo de este itinerario; sin embargo, es posible observarlos a lo largo de una estrecha franja que se extiende por la ladera norte del Carrascal de la Font Roja, así como al Oeste de la población de Cocentaina. Localmente las arcillas y yesos triásicos también pueden localizarse interestratificados en las margas Tap, correspondiendo en este caso a cuerpos resedimentados durante el Mioceno.

Estratigráficamente se puede reconocer un Mioceno inferior de calizas blancas que afloran en las proximidades del Mas de Serelles (Parada 6). Otros materiales permeables que es posible observar a lo largo del itinerario serían las calcarenitas y areniscas tortonienses y los conglomerados y lutitas messinienses, ambos corresponderían a un Mioceno superior. Los tramos intermedios entre los indicados anteriormente corresponderían a litologías margosas (facies Tap) que presentarían características impermeables en conjunto, aunque podrían intercalar niveles de caliza o calizas margosas.

Esta alternancia de materiales de baja o muy baja permeabilidad con otros de permeabilidad media, justifica la aparición de pequeñas surgencias como la Font de Serelles. Se trata de manantiales que, a pesar de encontrarse colgados respecto a la descarga de base de sus acuíferos, presentan un carácter permanente, aportando caudales durante todo el año.

Los materiales más recientes que se pueden observar en diversos puntos del itinerario corresponden a depósitos coluviales cuaternarios, caracterizados por la angulosidad de sus brechas y la homogeneidad de sus litologías (IGME. 1995).

### Flora y vegetación

La situación privilegiada de la sierra de Mariola favorece el desarrollo de una vegetación densa y estratificada, sobre todo en las umbrías. Los vientos cargados de humedad procedentes del Mediterráneo son canalizados por los valles litorales y subir en altura se produce la condensación, formando un mar de nubes que favorece la precipitación horizontal. Junto a estas condiciones microclimáticas, la accidentada topografía y la diversa litología, propician el desarrollo de diferentes tipos de formaciones forestales y bosques compuestos por un variado número de especies arbóreas, arbustivas y herbáceas.

Podemos encontrar buenos ejemplos de la vegetación potencial climática, representada en estas tierras por el carrascal e incluso por el bosque mixto mediterráneo en las zonas umbrías.

El carrascal se sitúa mayormente sobre los canchales estabilizados; dominado por la carrasca (*Quercus rotundifolia*), se enriquece en las umbrías con especies de árboles caducifolios como el fresno (*Fraxinus ornus*), el arce (*Acer opalus subsp. granatense*), el mostajo (*Sorbus aria*), el serbal (*Sorbus torminalis*) y el perennifolio tejo (*Taxus baccata*); algunos ejemplares milenarios de tejo se encuentran en la microreserva de la Teixera d'Agres, donde se puede observar el bosque relíctico de tejos mas meridional de Europa. Todas estas especies arbóreas aparecen acompañadas por un rico estrato arbustivo compuesto por durillos, ruscos, madreselvas, aladiernos, etc. y enredaderas como la hiedra y la madreselva.

Situado sobre las margas yesíferas del Triásico Keuper, donde existe mayor disponibilidad de agua, incluso se puede observar un quejigar o robledal dominado por el quejigo (*Quercus faginea*).

En la zonas donde el bosque mixto se ha degradado aparece la siguiente etapa de degradación (coscojar) caracterizada por coscojas (*Quercus coccifera*), torviscos, enebros, espinos negros, y espinares en las zonas más húmedas y sombreadas compuestos de zarzas, endrinos, espinos albares, rosales silvestres, etc.

Este carrascal alcanza su máximo esplendor en el Parque Natural de la Font Roja, y debería dominar la mayor parte del territorio valenciano. Sin embargo, la mayor parte de la superficie de la Sierra de Mariola está cubierta por un denso pinar de repoblación de pino carrasco (*Pinus halepensis*), mezclado con brezos, aliagas, romeros, jaras y muchas de las plantas aromáticas y medicinales.

La Sierra de Mariola destaca entre otros espacios naturales de la Comunidad Valenciana por su singularidad y diversidad florística. Más de 1400 especies de plantas superiores se han catalogado en Mariola con muchos endemismos iberolevantinos y setabenses. Adquieren especial relevancia aquellas especies con propiedades aromáticas y medicinales, utilizadas desde tiempos inmemoriales con fines farmacéuticos y gastronómicos, para la elaboración de bebidas alcohólicas y como condimento. En este sentido cabe destacar la famosa salvia endémica de Mariola (*Salvia blancoana* subsp. *mariolensis*), el romero, el tomillo, el rabo de gato, la manzanilla borde, el apreciado y escaso fresnillo o "timó real" (*Dictamnus hispanicus*), la pebrella (*Thymus piperella*), el espliego, el hipérico, el té de roca y un largo etcétera.

La Sierra de Mariola alberga 2 especies de flora Catalogadas (Catálogo Valenciano de especies de flora amenazada):

La lechetrezna de Sierra Nevada (*Euphorbia nevadensis* subsp. *nevadensis*) catalogada como Vulnerable (VU). Se trata de un endemismo ibérico que se extiende por el centro, sur y sudeste de la Península Ibérica. En la Comunidad Valenciana sus poblaciones quedan restringidas a la Sierras de Aitana y Mariola.

La orquídea mariposa (*Orchis papilionacea*) catalogada como En Peligro de Extinción (EPE). Especie dispersa por toda la Comunidad Valenciana en la que destacan 4 poblaciones en la Sierra de Mariola.

En las cotas más bajas de la sierra, en aquellas zonas donde el carboneo y la extracción de madera de carrasca han sido intensos, el carrascal ha sufrido una fuerte degradación. Este es el caso del paraje de Serelles, enclavado en el interior del Parque Natural de la Sierra de Mariola. Aquí, los pinares de pino carrasco actualmente existentes, son en gran parte el resultado de repoblaciones efectuadas sobre antiguas zonas de carrascal; así como recolonización natural del pinar en bancales cuyo uso agrícola se abandonó. Así, la vegetación dominante es un denso y adulto pinar dominado por Pinus halepensis, con un estrato arbustivo muy diverso, como se ha descrito anteriormente, con brezos, enebros, coscojas, aliagas, romeros, jaras y muchas de las plantas aromáticas y medicinales citadas. Como parte del estrato arbóreo y en algunos enclaves muy concretos, es posible observar ejemplares dispersos de carrasca, que nos indican cual fue la vegetación original anterior a las repoblaciones forestales. Posterior al incendio de 2012, se puede observar una recuperación mas o menos lenta del pinar y destacan las especies herbáceas y arbustivas germinadoras como las jara blanca (Cistus albidus), las aliagas (Genista scorpius y Ulex parviflorus), el romero (Rosmarinus officinalis), etc. y rebrotadoras como el lastón (Brachypodium retusum), la coscoja (Quercus coccifera) y el enebro (Juniperus oxycedrus). En algunas zonas del incendio de 2012, sin embargo, la regeneración de pinar está siendo de una densidad muy elevada y se están estudiando tratamientos de clareos en edad joven con el fin de evitar tener en plazo de 20-30 años un riesgo muy elevado de incendio y favorecer el crecimiento de los pies de pino que se dejan ya que el agua es un factor limitante en esta región.

Cabe mencionar el área de la Font del Serelles, donde se pueden observar varias especies de árboles como chopos, plátanos, pinos y otras especies, algunas de ellas introducidas en este área recreativa y que alcanzan un gran porte gracias a la humedad del enclave.

## Usos del suelo

El paisaje entendido como un elemento más del medio depende a su vez de múltiples factores ambientales como antrópicos, pero es la influencia del hombre la que tiene un mayor impacto sobre el mismo. Así es, que el paisaje ha ido variando fuertemente con el desarrollo de la sociedad, organizando el territorio en base a la obtención de los recursos y necesidades humanas. En la zona de Alcoi durante los siglos XVII y XIX existía una economía eminentemente agrícola que buscaba nuevas zonas de cultivo a costa de terrenos forestales y que se ayudaba de pequeños ganados que complementaban las rentas. Este tipo de economía tenía una ordenación característica, donde el territorio quedaba dividido en funcionalmente en fincas, que agrupaban las zonas agrícolas alrededor de las masías, como elemento constructivo agrícola principal. Otro de los elementos que ha condicionado el paisaje ha sido el cultivo en zonas con elevadas pendientes. Para que esto fuera posible, deteniendo los procesos erosivos, se procedía al abancalamiento de los terrenos y a la construcción de márgenes de piedra de mampostería en seco, lo que ha dado lugar a un paisaje característico con una ordenación territorial concreta.

Así mismo, en este tipo de economía, las zonas forestales eran utilizadas para la obtención de recursos como carbón, leñas, pastos o caza, cosa que unida a una mayor extensión de las zonas agrícolas limitaba la existencia de masas arboladas y zonas forestales, tal y como las conocemos hoy en día. Si se observan fotografías de principios del siglo pasado, se puede observar este aspecto, con la mayoría de las montañas que hoy vemos muy pobladas con vegetación rala y escasa.

Con el desarrollo industrial, se produce un abandono paulatino de las zonas agrícolas menos rentables, así como el abandono de aprovechamientos forestales en busca de mejores rentas. Esto produce por un lado la extensión de las masas y superficies forestales y por otro, el desarrollo de las comunidades vegetales, que quedan en algunos lugares sin ningún tipo de gestión. Cabe señalar que a partir de mediados del siglo XX también existe un programa de repoblaciones forestales a nivel estatal, que tiene incidencia en la zona de Alcoi logrando recuperar terreno para el bosque en zonas donde la recolonización natural hubiera sido difícil.

La primera ley de suelo en España data del 12 de mayo de 1956. Con ella ya existe un marco normativo moderno con consecuencias directas en la ordenación y gestión del territorio. En ella ya se introducen las primeras clasificaciones de suelo y condicionantes constructivos. Las leyes de suelo se suceden y las comunidades autónomas van adquiriendo competencias en la materia, hasta llegar a la actual Ley 5/2014, de 25 de julio, de Ordenación del Territorio, Urbanismo y Paisaje, de la Comunitat Valenciana. Toda esta normativa urbanística se plasma en los municipios mediante los Planes Generales de Ordenación Urbana, donde cada municipio en función de sus necesidades y criterios, procede a la ordenación de su territorio.

Entre las normativas existentes, las medioambientales y las de espacios naturales protegidos tienen también una gran influencia sobre la ordenación del territorio y el paisaje, anteponiendo criterios ambientales y conservacionistas en la gestión, a otros criterios, y preservando estas zonas de ciertos usos y desarrollos urbanísticos.

En definitiva, el paisaje y los usos del territorio, tal y como los conocemos hoy en día, son fruto de los usos y aprovechamientos pasados, situaciones sociales y de las distintas normativas que se han ido sucediendo, conformando y transformando nuestro entorno hasta la situación actual. La Sierra de Mariola fue declarada Parque Natural por el Decreto 3/2002, de 8 de enero de 2002, del Gobierno Valenciano, [2002/280] (DOGV nº 4167 de fecha 14.01.2002).

## Suelos

El relieve abrupto de la montaña alicantina tiene una gran influencia en los suelos, en las partes más altas predominan suelos esqueléticos, poco evolucionados o en algunos casos relictos donde los restos de horizontes argílicos se acumulan en oquedades de roca de naturaleza kárstica. En las partes medias predomina el constante rejuvenecimiento de los suelos por aportes y pérdidas por erosión. La orientación de las laderas es un factor relevante también, controlando a favor o en contra el balance hídrico del suelo, siendo las más desfavorecidas las orientadas a S y SE. La particularidad de los usos anteriores del suelo y los abancalamientos permiten tener un mayor espesor de suelo en estas estructuras, si bien es frecuente observar bancales semidestruídos y aparición de procesos erosivos. En las zonas bajas de las laderas, como es de esperar, tenemos suelos con mayor profundidad. Es fácil encontrar endopediones cámbicos, cálcicos, y petrocálcicos, en muchos casos sobre depósitos coluviales, y como epipediones, ócricos en la mayoría, pero donde las condiciones lo permiten (umbrías y zonas sin perturbaciones recientes), podemos encontrar horizontes móllicos, ricos en materia orgánica y saturados en bases (Sánchez Díaz et al., 2019).

Los materiales de origen son principalmente de naturaleza carbonatada, calizas, margocalizas y margas son las más dominantes en la zona de la Sierra de Mariola. Esto le da, especialmente en los suelos jóvenes, un pH básico y riqueza en carbonatos, salvo en algunos

alfisoles antiguos donde nos podemos encontrar que los carbonatos prácticamente han desaparecido. El régimen de humedad del suelo en la zona es Xérico.

Los suelos más dominantes según la Soil Taxonomy (Soil Survey Staff, 2014), son Entisoles (Xerorthents), Inceptisoles (Haploxerepts y Calcixerepts) y como indicamos, cuando las condiciones han permitido el desarrollo de un horizonte móllico encontramos Mollisoles (Calcixerolls y Haploxerolls).



Figura 2. Uno de los perfiles descritos y analizados en la zona y clasificado como un Haploxerloll lítico (SSS, 2014)

En la zona de estudio de la saca de madera que visitaremos en una de las paradas (parada 4), el suelo se clasifica como un Xerorthent típico, y está desarrollado sobre margas; tanto el suelo como el material parental son muy vulnerables a los procesos de erosión y degradación. El suelo tiene un 4,6% de contenido en materia orgánica en los primeros 5 cm, un 44% de carbonatos, y su textura se compone por 45% arena, 39% limo y 17% arcilla. El pH del suelo es ligeramente alcalino (8,3) y presenta una conductividad eléctrica de 172,5  $\mu$ S/cm. En el recorrido de la excursión, haremos también un par de paradas en dos perfiles que tenemos descritos y analizados (más información al final de esta guía).

#### Incendios y manejos

La zona ha sufrido dos incendios recientes, uno en 1994, principalmente en la zona de Gormaig, de 380 ha y el más reciente, el 12 de julio de 2012, el cual afectó a 545,9 ha en el Parque Natural de la Sierra de Mariola, siendo considerado como gran incendio forestal (GIF) al superar las 500 ha de envergadura y convirtiéndose en el mayor incendio sufrido por la zona desde la declaración como Parque Natural. En su mayor parte el incendio de 2012 afectó a la zona ya quemada en 1994, sin embargo algunos rodales quemados en 1994 se salvaron de este último y nos han servido para realizar estudios comparativos.

El manejo post-incendio más frecuente ha sido la extracción de madera quemada. Tras el incendio de 2012 y gracias a la estrecha colaboración con el Ayuntamiento de Alcoi, hemos podido realizar un estudio sobre el impacto que tiene este tratamiento en las propiedades del suelo y la recuperación de la cubierta vegetal. Por otro lado se estudió el efecto de la aplicación de acolchados (paja y astilla de madera), este último tratamiento se ha venido realizando también en la línea de divulgación con voluntariado, actividades con escuelas, el día del árbol etc. como detallaremos a continuación.

#### Investigación y divulgación

En cuanto a **investigación**, la línea principal se ha centrado desde Febrero de 2013 en el estudio y seguimiento a medio plazo del impacto que ha tenido la saca de madera quemada sobre el suelo, siempre comparando zona sometida al tratamiento frente a zona control (quemada sin extracción de madera quemada). Con el fin de obtener unos resultados comparables, todas las parcelas de los dos tratamientos se marcaron en zonas con condiciones similares en cuanto a orientación, pendiente, vegetación previa, etc.

El primer muestreo se hizo inmediatamente después de que tuviese lugar la saca de madera (2013; Figuras 3 y 4), y a partir de ese momento cada seis meses para después espaciarlos a un muestreo anual. Suman hasta el momento ocho muestreos, realizados en las proximidades de las áreas marcadas para respetar la cobertura vegetal. Se han estado tomando muestras de los primeros centímetros de del horizonte mineral superficial. La profundidad a la que se toman las muestras (0-5 cm) viene determinada por un promedio de la profundidad a la que se estima que afecta este tipo de tratamiento (saca de madera). La profundidad a la que se ve afectado el suelo en determinadas zonas donde los troncos han sido arrastrados y apilados fue mayor, pero no es representativa; por ello, estas zonas fueron evitadas a la hora de instalar las parcelas, de manera que se eligieron las áreas más representativas del tratamiento realizado.



**Figura 3**. Ejemplo de parcelas en zona de estudio. Imagen izquierda: parcela marcada para el seguimiento en zona de saca de madera, derecha: parcela de la zona control (quemado sin intervención de extracción de madera) (fotografías: J. Mataix-Solera, 2013)



**Figura 4**. Imagen izquierda: zona afectada por el incendio de 2012 justo tras actuaciones de extracción de madera quemada a los 6 meses del incendio. Imagen derecha: cárcava formada en la zona tras una lluvia torrencial de 50 mm en una tarde a los pocos meses de la extracción de madera quemada (fotografías: J. Mataix-Solera, 2013)

Los parámetros que se han venido analizando habitualmente han sido: repelencia al agua, capacidad de campo, contenido de materia orgánica, nitrógeno, fósforo asimilable, biomasa microbiana, respiración edáfica y estabilidad de agregados. Puntualmente y en colaboración con otros colegas se han realizado otros análisis más específicos, microbiológicos

(PLFAs, ADN, etc.), así como algunos experimentos y estudios enfocados al comportamiento hidrológico de los suelos con simuladores de lluvia (Figura 5), infiltrómetros de campo y toma de muestras inalteradas para estudios de curvas de retención hídrica (Mataix-Solera et al., 2016a y b). En el texto de esta guía resumimos los resultados y conclusiones más relevantes de estos estudios, si bien, en anexos se pueden consultar las publicaciones más relevantes que tenemos hasta la fecha en revistas y congresos.



Figura 5. Simulador de lluvia portátil utilizado en el estudio, diseñado por Kamphorst (1987) (fotografías: Pablo Aranáiz, 2015)

Durante 2018 y 2019 se han empezado a estudiar nuevos tratamientos en la zona. Uno de clareos en la zona quemada de 2012 y otro en una zona que se quemó en 1994 y se salvó del de 2012. La finalidad es comprobar si estos tratamientos tienen un efecto negativo en el suelo. Consideramos que son necesarios cuando el regenerado de pinar es muy denso y sería más fácil y viable realizarlos en estadios tempranos de recuperación, cuando los pinos tienen 4-5 años, pero desconocemos todavía qué impacto pueden tener en el suelo. Para ello en la zona control del incendio de 2012 se han establecido parcelas en las que se ha dejado solo un pie por cada 4 m<sup>2</sup>, y en una zona quemada en el año 1994 y no afectada por el incendio de 2012, el Ayuntamiento ha realizado trabajos silvícolas de clareos (Figura 7), dejando una zona sin tratar para tener un control sin tratamiento y poder comparar los resultados.

Por último se está estudiando también el papel de los briófitos en las propiedades del suelo ya que en la zona de estudio donde están las parcelas de saca y control se observó una importante presencia de estos organismos cubriendo algunas zonas (Figura 8) y hemos considerado de alto interés su estudio. Se realizaron medidas en campo de cobertura, y experimentos de infiltración, compactación y repelencia al agua tanto en suelo desnudo como sobre y debajo de los musgos en un total de 100 puntos y se tomaron muestras en todos estos puntos para su análisis en laboratorio. También se ha estudiado si hay cambios en las comunidades microbianas en los suelos influidas por la presencia de briófitos, trabajo que se presenta como comunicación en este congreso y que se puede consultar también aquí en anexo.

Tras 5 años desde la realización de la extracción de madera quemada (muestreo de 2018) se constata que siguen existiendo diferencias significativas en los parámetros analizados estando estos en la zona de la Saca con valores por debajo respecto a la zona Control. La cierta recuperación que se observó el año 2016 se vio frenada por los intensos episodios de precipitaciones ocurridos a finales de ese año que causaron erosión incluso en la zona control (quemada sin tratamiento de saca de madera). Además, todos los datos de los parámetros estudiados sobre la flora (riqueza, abundancia y diversidad) y la vegetación durante los dos primeros años nos indicaron un efecto negativo de la "saca" sobre misma, provocando una disminución del banco de semillas y una desaparición de especies rebrotadoras (Jara-Navarro, 2015).



**Figura 6.** Imagen izquierda: zona afectada por el incendio de 2012 y sometida a la extracción de madera quemada, foto tomada a los 3 años del incendio. Imagen derecha: muestra de suelo quemado sometido a la extracción de madera quemada, izqda., y de la zona control, dcha. (quemado sin intervención de extracción de madera) (fotografías: J. Mataix-Solera, 2015)



Figura 7. Imagen izquierda: recogida de muestras (Muestreo 8) en una de las parcelas de la zona Control. Imagen derecha: zona afectada por el incendio de 1994 tras ser sometida al tratamiento de clareo (fotografías: J. Mataix-Solera, 2018)



Figura 8. Ejemplo de la presencia de briófitos (fotografías: J. Mataix-Solera, 2018)

Como conclusiones, se puede afirmar que en este tipo de suelo, el cual es muy vulnerable a la erosión y a la degradación, la saca de madera tiene un efecto negativo en el ecosistema (García-Orenes et al., 2017; Pereg et al., 2018). Esta degradación se mantiene en el tiempo, reduciéndose el ritmo de recuperación. También hemos podido comprobar que episodios de lluvias intensas pueden tener un fuerte impacto en este suelo, como demuestran los resultados obtenidos. Esto demuestra que esta zona, 6 años después del incendio, sigue siendo vulnerable a la erosión y a la degradación, y que todavía serían efectivas medidas de

conservación del suelo como la protección con acolchados (Mataix-Solera et al., 2016a y b). Dado que los suelos bajo musgo han mostrado mejores condiciones de estabilidad, fertilidad y respuesta microbiológica (García-Carmona et al., 2020), y su presencia fue menor en la zona de saca, este manejo sigue teniendo un efecto a día de hoy sobre los suelos. La saca de madera fue una práctica muy agresiva con el suelo, pero el desarrollo de costras biológicas sobre el suelo estaría protegiendo el mismo a medio plazo de una mayor degradación.

A la vista de estos resultados, recomendamos que para una mejor gestión forestal se tenga en cuenta el desarrollo de las costras biológicas, y en especial los musgos, con el propósito de no afectar a su desarrollo en los manejos post-incendio que se planeen. Aún así, es necesario una investigación más profunda para comprobar si ocurre los mismo bajo otros tipos de suelos y el efecto de otros manejos post-incendio.

En cuanto a asesoramiento y **divulgación** del estudio y los resultados, además de los trabajos presentados en congresos y los artículos científicos, desde 2015, se han venido realizando actividades de divulgación in situ en colaboración con el ayuntamiento de Alcoi. En 2015 al ser el Año Internacional de los Suelos declarado por la FAO, se realizó una actividad en coordinación con todas las escuelas asociadas a la UNESCO de Alcoi. Participaron unos 80 estudiantes y consistió en una actividad de campo utilizando un simulador de lluvia sobre parcelas de la zona afectada por el incendio, y donde por grupos, los estudiantes realizaron diversos manejos (extracción de vegetación, compactación del suelo, aplicado de acolchados, etc.). Tras los tratamientos se fue pasando el simulador de lluvia y pudieron comprobar con los resultados, la importancia del manejo post-incendio en la conservación del suelo y el agua.



Figura 9. Imágenes de diversas actividades llevada a cabo el día del árbol durante estos años (fotografías: J. Mataix-Solera, 2016 - 2018)

En los últimos años se han venido diversificando las actividades realizadas para conmemorar el día del árbol, siempre adaptándonos a la evolución del paisaje tras el fuego. Por un lado consideramos continuar con la actividad realizada en años anteriores, la de cuidar y proteger el suelo en las zonas donde la cobertura vegetal sigue siendo muy escasa mediante la aplicación de un acolchado "mulch" de astilla de madera. Pretendíamos protegerlo y de esta manera "cuidar" la vegetación que está recuperándose y evitar que se degrade más el suelo y el ecosistema, al mismo tiempo que hacer una labor divulgativa en este sentido. Por otro lado en algunas zonas donde la densidad de regenerado de pino era excesiva se decidió que sería

bueno realizar algunos clareos y eliminar algunos pinos para disminuir la competencia y dejar que los que queden puedan desarrollarse y crecer mejor con el objetivo de buscar futuros bosques más resilientes con el fuego. También con los más pequeños se han realizado en algunos años talleres sobre el papel de la cobertura vegetal en la erosión del suelo (Figura 9).

En 2017 también se instaló en una zona con buena visibilidad y afluencia de senderistas un panel divulgativo sobre los efectos del fuego y de los manejos post-incendio (Figura 10), y en 2019 también se colaboró con el ayuntamiento en la elaboración de un cuadernillo que se repartió entre los asistentes y también se imprimieron copias de un díptico con la misma información que hay en el panel instalado en al zona.



Figura 10. Imagen izquierda: panel divulgativo instalado en la senda que recorre Serelles. Imagen derecha: portada y contraportada del cuadernillo elaborado sobre los incendios forestales, recuperación y cuidado (fotografías: J. Mataix-Solera, 2017)

La zona de estudio supone un laboratorio al aire libre, que por su ubicación (cercana a los núcleos de Alcoi y Cocentaina), y disponer de una senda muy transitada por excursionistas tiene un potencial enorme para la transferencia directa de resultados y divulgación del impacto del fuego y manejos post-incendio. Esto lo hemos podido ir constatando en las distintas actividades que hemos ido realizando como en el día del árbol y consideramos por tanto que sería interesante continuar con labores de divulgación con grupos sociales, tal y como se ha venido realizando todos estos años.

Durante la excursión se realizarán paradas en estas zonas de estudio y donde se han llevado a cabo las actividades divulgativas.

## Perfiles de suelos

## Perfil 1



#### **Características Generales**

Localización	Sierra de Mariola
Coordenadas	38° 43′ 51,17′′ N
	0° 29´ 6,58´´ W
Altitud	865 m
Orientación	NE
Pendiente	0 - 2 %
Relieve	Antiguo bancal
Erosión	Sin erosión
Posición topográfica	Mitad de la ladera
Material original	Margas
Vegetación o uso	Forestal. Parque Natural



Clasificación tentativa: - WRB: Hypereutric Calcaric Regosol (Siltic, Escalic, Humic) (IUSS, 2015) - Soil Taxonomy: Xerorthent típico (SSS, 2014)

#### Datos morfológicos

Horizonte	Prof. (cm)	Descripción
Α	0 - 8	Color en húmedo y en seco gris parduzco claro (2.5Y 6/2); textura franco limosa;
		fragmentos gruesos en un 10% del horizonte; estructura granular, de desarrollo moderado; extremadamente calcáreo; límite inferior neto y plano
ABwb	8 - 19	Color en húmedo pardo grisáceo (2.5Y 5/2) y gris parduzco claro en seco (10YR 6/2);
		textura franco limosa; fragmentos gruesos en un 5%; estructura subangular, de
		desarrollo moderado; extremadamente calcáreo; límite inferior neto y plano
BC	19 - 40	Color en húmedo y seco gris parduzco claro en seco (2.5Y 6/2); textura franco limosa;
		fragmentos gruesos en un 15%; estructura en bloques angulares, de desarrollo fuerte;
		extremadamente calcáreo; límite inferior gradual y plano
С	+ 40	Saprolito deleznable originado de margas

#### Datos analíticos

Horizonte	рН	<b>CE</b> (dS m <sup>-1</sup> )	CaCO <sub>3 eq</sub> (%)	MO (%)	<b>P</b> (mg Kg⁻¹)	N (%)	<b>EA</b> (%)
Α	8,4	0,122	62,9	3,39	2,70	0,10	65,5
ABwb	8,4	0,116	64,6	3,67	2,62	0,13	n.d.
BC	8,2	0,192	69,2	3,20	2,68	0,10	n.d.

Horizonte	Granulometría (%)			Cationes de cambio (Cmol <sub>c</sub> Kg <sup>-1</sup> )					
	Arena (%)	Limo (%)	Arcilla(%)	Ca <sup>2+</sup>	Mg <sup>2+</sup>	K⁺	Na⁺	<b>CIC</b> (Cmol <sub>c</sub> Kg <sup>-1</sup> )	<b>V</b> (%)
Α	27,9	62,1	10,0	11,47	0,98	0,60	0,07	13,12	100
ABwb	26,0	68,1	5,9	12,38	0,96	0,50	0,07	13,93	100
BC	29,8	64,3	5,9	14,99	0,92	0,42	0,08	16,41	100

CE: Conductividad Eléctrica; MO: Materia orgánica; EA: Estabilidad de agregados, arena (2-0.05 mm); limo (0.05 – 0.002); arcilla (< 0.002 mm), CIC: Capacidad de intercambio catiónico, V: saturación en bases, n.d.: no determinado

### Perfil 2



#### **Características Generales**

Localización	Sierra de Mariola
	"Collado de la Zapata"
Coordenadas	38° 44′ 3,34′′ N
	0° 29′39,23′′ W
Altitud	1100 m
Orientación	NE
Pendiente	0 - 2 %
Relieve	Colina de gradiente medio
Erosión	Sin erosión
Posición topográfica	Cresta (cumbre)
Material original	Calcarenitas
Vegetación o uso	Forestal. Parque Natural



Clasificación tentativa: - WRB: Akroskeletic Leptic Chernic Rendzic Phaeozem (Endoraptic) (IUSS, 2015) - Soil Taxonomy: Haploxeroll lítico (SSS, 2014)

#### Datos morfológicos

Horizonte	Prof. (cm)	Descripción
Ah1	0 - 10	Color en húmedo pardo grisáceo muy oscuro (10YR 3/2), en seco pardo grisáceo muy
		oscuro (10YR 4/2); textura franco arcillosa; fragmentos gruesos en un 10%;
		estructura granular, de desarrollo fuerte; extremadamente calcáreo; límite inferior neto y fracturado.
Ah2	10 - 28	Color en húmedo pardo grisáceo muy oscuro (10YR 3/2), en seco pardo grisáceo muy
		oscuro (10YR 4/2); textura franco arcillosa; fragmentos gruesos en un 15%; estructura
		granular, de desarrollo moderado; extremadamente calcáreo; límite inferior neto e
		irregular
В	28 - 47	Color en húmedo pardo (10YR 5/3), en seco gris parduzco claro (10YR 6/2); textura
		franco limosa; fragmentos gruesos en un 60%; estructura en bloques subangulares,
		de desarrollo débil; extremadamente calcáreo; límite inferior abrupto e irregular
R	47- 70	Calcarenitas con intercalaciones de calizas margosas muy poco penetrable
2C	70 -	Margas saprolíticas deleznables

## Datos analíticos

Horizonte	рΗ	<b>CE</b> (dS m <sup>-1</sup> )	CaCO <sub>3 eq</sub> (%)	MO (%)	<b>P</b> (mg Kg⁻¹)	N (%)	<b>EA</b> (%)
Ah (0-28cm)	8,2	0,134	60,4	5,81	2,29	0,28	88,7
В	8,3	0,134	73,6	2,54	2,26	0,13	n.d.

Horizonte	Granulometría (%)			Cationes de cambio (Cmol <sub>c</sub> Kg <sup>-1</sup> )					
	Arena (%)	Limo (%)	Arcilla(%)	Ca <sup>2+</sup>	Mg <sup>2+</sup>	K⁺	Na⁺	CIC (Cmol <sub>c</sub> Kg <sup>-1</sup> )	<b>V</b> (%)
Ah (0-28cm)	20,3	45,6	34,2	17,14	0,95	0,53	0,06	21,85	85,5
В	27,8	51,9	20,3	12,38	0,79	0,12	0,07	13,37	100

CE: Conductividad Eléctrica; MO: Materia orgánica; EA: Estabilidad de agregados, arena (2-0.05 mm); limo (0.05 – 0.002); arcilla (< 0.002 mm), CIC: Capacidad de intercambio catiónico, V: saturación en bases. n.d.: no determinado

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# ANEXO

Trabajos publicados en revistas o actas de congresos

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## Science of the Total Environment



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# Effects of salvage logging on soil properties and vegetation recovery in a fire-affected Mediterranean forest: A two year monitoring research



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#### HIGHLIGHTS

#### GRAPHICAL ABSTRACT

- Mediterranean soils developed over marls are very vulnerable to degradation after wildfire.
- Post-fire salvage logging affects soil quality over short- and medium-terms.
- Plant recovery is also affected by salvage logging treatments after forest fire.
- Reduced plant cover and lower soil quality could increase soil erosion rates.



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#### ABSTRACT

Post-fire management can have an additional impact on the ecosystem: in some cases, even more severe than the fire. Salvage logging (SL) is a common practice in most fire-affected areas. The management of burnt wood can determine microclimatic conditions and seriously affect soil properties. In some cases, the way of doing it, using heavy machinery, and the vulnerability of soils to erosion and degradation can make this management potentially aggressive to soil. Research was done in "Sierra de Mariola Natural Park" (E Spain). A forest fire (>500 ha) occurred in July 2012. In February 2013, SL treatment was applied in a part of the affected forest. Plots for monitoring this effect were installed in this area and in a similar nearby area where no treatment was done, used as control (C). Soil samplings were done immediately after treatment and every 6 months during two years. Some soil properties were analysed, including organic matter (OM) content, nitrogen (N) available phosphorous (P) basal soil respiration (BSR), microbial biomass carbon (C<sub>mic</sub>), bulk density (BD), water repellency (WR), aggregate stability (AS) and field capacity (FC). SL treatment caused an increase in BD, a decrease of AS, FC, OM and N. In the control area, in general the soil properties remained constant across the 2 years of monitoring, and the microbial parameters (BSR and Cmic), initially affected by the fire, recovered faster in C than in the SL area. Plant recovery also showed some differences between treatments. No significant differences were observed in the number of plant species recorded (richness) comparing C versus SL plots, but the number of individuals of each species (evenness) was significantly higher in C plots. In conclusion, we can affirm that for the conditions of this study case, SL had a negative effect on the soil-plant system.

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#### 1. Introduction

Forest fires are part of the natural dynamic of the terrestrial ecosystem (Le Houerou, 1977; Naveh, 1975), and are also reflected in the fossil record and the great number of species adaptation due to this natural disturbance. The Mediterranean ecosystems have been and currently are very influenced by this phenomenon, as fire is a recurrent fact in the Mediterranean area, and one of the main causes of changes in these ecosystems. Fire induces changes in soil properties (e.g.: Andreu et al., 1994; Cerdà, 1996; Certini, 2005; Llovet et al., 2008), and can have an impact on soil productivity of burnt areas (Robichaud, 2009) especially when they are affected by huge and high intensity fires such as those that are frequent in some summer seasons under extreme weather conditions.

Post-fire management can have a negative impact on the soils being in some cases even more severe than the fire itself. Salvage logging (SL) is a common management technique in fire-affected areas, comprising the extraction of the burnt wood and in many cases using heavy machinery and dragging the trunks over soil, leading to a consequent increase in its vulnerability to erosion and soil degradation (Mataix-Solera et al., 2015, 2016).

González-Ochoa et al. (2004), Martínez-Sánchez et al. (1999), Pausas et al. (2004), Vega et al. (2008), and Griffin et al. (2013), among others, studied the vegetation dynamic in areas affected by fires and where SL treatments were applied. All of them concluded that to a greater or lesser degree there was a negative influence on plant recovery. Wagenbrenner et al. (2016), Morgan et al. (2014) and Sexton (1998) observed, reduced vegetation cover in areas where SL treatment was used compared to unlogged areas. Vegetation recovery after post-fire SL might have been hampered by the soil compaction (Page-Dumroese et al., 2006) or lower water availability (Marañón-Jiménez et al., 2013) caused by the equipment traffic. Numerous studies have demonstrated that soil compaction in forested environments can also persist for several decades, which can impede plant development for prolonged time periods (Wert and Thomas, 1981; Froehlich et al., 1985; Vora, 1988; Reisinger et al., 1992; Brevik, 2013).

Previous studies have shown that post-fire logging can increase soil disturbance and erosion (Klock, 1975; McIver and Starr, 2001; McIver and McNeil, 2006; Slestak et al., 2015; Wagenbrenner et al., 2015; Peterson and Dodson, 2016), alter the cover and composition of recovering native vegetation (Stuart and Grifantini, 1993; Purdon et al., 2004; Keyser et al., 2009; Ritchie et al., 2013), damage natural tree regeneration (Keyser et al., 2009) and increase surface woody fuels within 2–4 years after fire and logging (Donato et al., 2006).

There are several factors contributing to the increased runoff rates and erosion after SL treatment. Wagenbrenner et al. (2016) attributed the increase in runoff after logging equipment traffic to lower infiltration rates resulting from reduced micro and macro soil porosity (Ares et al., 2005; Horn et al., 2004; Startsev and McNabb, 2000), associated it with an increase in soil compaction. Wilson (1999), in his rainfall simulation experiments found that post-fire SL did increase surface runoff and erosion in part caused by the disruption of the biotic crust by heavy equipment. However, in NE Spain Marques and Mora (1998), observed that the sediment yields in burned areas as a consequence of the SL treatment were moderately low, and recently in NW Spain, Fernández and Vega (2016) didn't find any detrimental effect of SL treatment compared to natural regeneration. These contradictory results could be due to factors such as type of soil, when and how the SL treatment is carried out, and meteorological conditions which can be decisive.

Little is known about what effect this kind of treatment has in soil properties and how this can affect the ecosystem response to the fire and post-fire managements. Marañón-Jiménez et al. (2011) observed a decrease in soil respiration after salvage logging treatment, and Serrano-Ortiz et al. (2011) a negative impact for potential carbon sequestration. Post-fire SL treatment reduces the vegetation cover (Serrano-Ortiz et al., 2011; Wagenbrenner et al., 2016), and affects soil micro-climate (Lindenmayer and Noss, 2006), decomposition, and ecosystem carbon storage capacity. Moreover, post-fire SL can reduce organic matter input to the soil by removing the standing dead trunks that would eventually fall and contribute to soil organic carbon storage (Smith et al., 2000; DeLuca and Aplet, 2008; Moroni et al., 2010; Seedre et al., 2011). All these factors can also provoke a fast response in microbial properties, producing important changes in the content of microorganisms and their activity (Burton et al., 2000; Nadelhoffer, 2000; Tang et al., 2003).

In this research we studied the immediate, short and medium-term effect (2 years of monitoring) of a SL treatment applied in a recently burned area (6 months after fire) with a soil vulnerable to degradation in physical, chemical and microbial soil properties, and also in the restoration of vegetation recovery, by comparison with no treatment (control) to study whether this management can have an impact on soil quality and the magnitude of these changes.

#### 2. Materials and methods

#### 2.1. Study site

The study area is located in "Sierra de Mariola Natural Park" in Alcoi, Alicante (E Spain). Coordinates of the study area are 38°43′59″N, 0°29′ 16"W. This area has a Mediterranean climate with 3-4 months of summer drought, usually from late June till September. This climate is characterised by a dry-hot summer and a wet-warm spring, autumn and winter. The annual average precipitation is 490 mm, which falls mainly in October–November (maximum rainfall in October, 71 mm). Mean monthly temperature is 14.8 °C (summer: 22.7 °C, winter: 7.9 °C). The forest is composed mainly of Pinus halepensis trees of around 40 years old with an understory of typical Mediterranean shrubs species such as Quercus coccifera, Rosmarinus officinalis, Thymus vulgaris, Brachypodium retusum, etc. The soil is classified as a Typic Xerorthent (Soil Survey Staff, 2014) developed over marls with a low depth, very vulnerable to erosion and degradation processes, with 4.6% of organic matter content in first 5 cm soil depth, a loam soil texture with 45, 39 and 17% of sand, silt and clay respectively, and 44% of carbonates.

A forest fire of moderate severity occurred in July 2012 affecting a total of 546 has. Six months after the forest fire, in February 2013, salvage logging (SL) treatments consisting of a complete extraction of the burned wood using heavy machinery was applied in a part of the affected forest. In the pictures of Fig. 1 an example of specific areas where logs were gathered and dragged onto the road during and just after the extraction of wood can be observed.

# 2.2. Experimental design, vegetation monitoring, soil samplings and analysis

Three plots of 4 m<sup>2</sup> for monitoring this effect were installed in this area and another three in a similar nearby area where no treatment was done, which were then used as control (C) for comparison. All plots in both treatments were chosen with the same aspect and slope for comparable results (Fig. 2). The six plots were monitored for plant recovery. In these plots, three seasonal periods were observed, autumn 2013, spring and autumn 2014. The following parameters were determined: *Richness* (number of plant species for each plot), *Evenness* (number of individuals of each species) and *Diversity*. The Shannon-Weaver (H') index was calculated as a measure of diversity, as it combines two components of diversity, i.e., species richness and evenness. It is calculated from the equation  $H' = -\Sigma pi(\ln pi)$ , where pi is the proportion of individuals found in the *i*th species.

Soils samplings were done immediately after post-fire treatments and every 6 months, until a total of five soil samplings were made in areas near the plots for plant recovery. Three soil samples (0-5 cm depth) per plot were collected from the A soil mineral horizon (n = 9



Fig. 1. Examples of salvage logging (SL) treatments (left Picture) and how the study area looked just after SL treatments, especially where logs are gathered and dragged onto the road (right picture). Photos: J. Mataix-Solera, 2013.

samples per treatment and per sampling; total n = 135 samples). The election of the depth of soil sampling was decided after estimations of the averaged mineral soil depth affected by the SL treatments. In some specific areas where logs were gathered and dragged, the affected soil depth was higher, but not representative. These specific areas were avoided to install the plots and all of them were installed in the most representative places after the treatment.

For every soil sample an aliquot was kept under 4 °C to measure the microbiological parameters, and the rest of the soil sample was dried at room temperature and sieved apart between 4 and 0.25 mm for Aggregate Stability (AS) tests and the remaining 2 mm for the rest of the physico-chemical analysis. In all the samples the organic matter content (OM), aggregate stability (AS), soil water repellency (WR), bulk density (BD), Kjeldahl N, available phosphorus (P), field capacity (FC), basal soil respiration (BSR) and microbial biomass carbon content (Cmic) were determined. Soil OM was determined by the potassium dichromate oxidation method (Nelson and Sommers, 1982). AS was measured with the method of Roldán et al. (1994), based on the method of Benito and Díaz-Fierros (1989). This method examines the proportion of aggregates that remain stable after a soil sample (sieved between 4 and 0.25 mm) is subjected to an artificial rainfall of known energy  $(270 \text{ Jm}^{-2})$ . Persistence of soil water repellency (WR) was assessed by the Water Drop Penetration Time (WDPT) test (Wessel, 1988). Approximately 10 g of air-dried sample (<2 mm) was placed into separate plastic dishes (diameter 50 mm), and exposed to a controlled laboratory atmosphere (20 °C, 50% relative humidity) for 24 h to eliminate potential effects of any variations in preceding atmospheric humidity on WR (Doerr et al., 2002). The average time for triplicate drops is taken as the WDPT value of a sample, and then classified according to Bisdom et al. (1993) and Doerr et al. (1998), as follows: wettable (WDPT < 5 s), slightly water repellent (WDPT: 5–60 s), strongly water repellent (WDPT: 60-600 s), severely water repellent (WDPT: 600-3600 s), and extremely water repellent (WDPT > 3600 s).

Total nitrogen was determined by the Kjeldahl method (Bremner and Mulvaney, 1982). Available phosphorus was determined by the Burriel-Hernando method (Díez, 1982). Field capacity was assayed by the method of Forster (1995). C<sub>mic</sub> was determined by the fumigationextraction method (Vance et al., 1987). The basal respiration of soil was measured in a multiple sensor respirometer (Micro-Oxymax, Columbus, OH, USA).

In the case of bulk density we performed soil samplings more frequently during the first year (one per month) trying to get more information as to whether soil compaction as a consequence of treatment could happen in the short-term. Bulk density was measured using undisturbed soil cores of 100 cm<sup>3</sup>.

#### 2.3. Statistical analyses

The fitting of the data to a normal distribution for all soil properties was checked with the Kolmogorov-Smirnov test at p < 0.05. To compare post-fire treatments effect (SL versus C), t-test for independent samples, for every one of the soil samplings was used; also a one-way ANOVA was carried out to know the differences between samplings (temporal changes) for each one of the two treatments. The separation of means was carried out according to the average post-hoc Tukey test p < 0.05, assuming equal variance. Redundancy analysis (RDA) was used to examine the relationship between the soil treatment and soil characteristics. Soil physical, chemical and microbial properties were tested for significant contributions to the variation data using the Monte Carlopermutation test (p < 0.05). Only the soil properties that were significantly correlated with factors in the RDA were included. Vectors represent soil properties. Vectors of greater magnitude that form smaller angles with an axis are more strongly correlated with that axis. All statistical analysis was performed with the SPSS program (Statistical Program for the Social Sciences 18.0). RDA was performed using CANOCO for Windows v. 4.5.3



Fig. 2. Examples of plots marked for monitoring the salvage logging (SL) effects on soil and vegetation. Left picture: plot installed in SL area, right picture: control plot in untreated area. Photos: J. Mataix-Solera, 2013.



Fig. 3. Temporal changes in soil aggregate stability (AS) from untreated-control (C) area versus salvage logging (SL) treated area during the 2 years of study. Different letters above the bars indicate significant differences between soil sampling periods (1–5), in capital and bold for SL treatment.

#### 3. Results

#### 3.1. Soil physical properties (BD, AS, WR and FC)

One of the physical effects of SL was compaction of the soil surface layer, indicated by an increasing trend of BD and a decrease of AS. Bulk density of this type of soil is relatively low since the parent material (marls) has a low real density. In the control area the BD varied between  $0.72 \pm 0.08$  to  $0.78 \pm 0.07$  g cm<sup>-3</sup> and without statistical differences throughout the whole study period. However, in the area with SL treatment a progressive tendency to increase was measured with an initial value of  $0.62 \pm 0.06$  for the first soil sampling, to  $0.83 \pm 0.09$  g cm<sup>-3</sup>

at the end of the study period, with statistical differences (p < 0.05) two years after the treatment, indicating a compaction of soil.

In Fig. 3, we can observe the evolution of AS for both treatments. After the fire, the percentage of AS was high, around 90%. SL treatment clearly provoked a decrease in AS (around 20%) that was observed 6 months after treatment and this difference was maintained until the end of the study. The control soils were able to maintain the same level of AS during the whole study period. This property is closely related with BD, and due to the SL treatment, the destruction of part of the aggregates contributed to clog soil pores and therefore increased the BD.

The field capacity shows high variability in values but in general we observed lower mean values in the SL area than in the C plots (p < 0.05)



Fig. 4. Temporal changes of field capacity (FC) from untreated-control (C) area versus salvage logging (SL) treated area during the 2 years of study. Different letters above the bars indicate significant differences between soil sampling periods (1–5), in capital and bold for SL treatment.



Fig. 5. Temporal changes of soil organic matter content (OM) from untreated-control (C) area versus salvage logging (SL) treated area during the 2 years of study. Different letters above the bars indicate significant differences between soil sampling periods (1–5), in capital and bold for SL treatment.

#### Table 1

Differences between treatments for every sampling along the experiment. Significant at: \*p < 0.05, \*\*p < 0.01; \*\*\*p < 0.001; ns: not significant (p > 0.05).

Parameter	Sampling 1	Sampling 2	Sampling 3	Sampling 4	Sampling 5
AS	ns	***	***	***	***
FC	**	ns	**	***	**
Р	ns	ns	**	ns	***
OM	ns	ns	***	***	***
Ν	ns	ns	**	***	***
BSR	ns	**	ns	**	***
C <sub>mic</sub>	ns	*	***	***	***

AS: aggregate stability; FC: field capacity; P: available phosphorous; OM: organic matter content; N: nitrogen; BSR: basal soil respiration, C<sub>mic</sub>: microbial biomass carbon.

for most of soil samplings (Fig. 4). Soil WR was not detected in any soil sample at any period of soil sampling (WDPT < 5 s).

#### 3.2. Soil organic matter content, nitrogen and available phosphorous

As we can see in Fig. 5, the OM content in the SL area shows a significant and important progressive decrease during the whole study period, reaching less than half of the initial content in the last sampling. We also found statistically significant differences in OM content between treatments for the three last samplings (p < 0.001; Table 1). The control area did not show variations for this parameter, keeping the initial soil OM content constant during the whole study period (Fig. 5).

The behaviour of N is similar to the OM content. The area that has suffered the wood extraction showed a significant decrease of the N content (50%) during the study period. Soil of control plots has kept its N content during the first samplings increasing it significantly from the third sampling. We found significant differences between the two treatments in last three samplings after SL (Fig. 6 and Table 1). Fig. 7 shows the evolution of available P during the study period, there were almost no differences for this parameter between treatments especially at the start of the study, although some lower mean values for SL treatment were obtained from the third soil sampling but with high variability of data. The soil of C area kept the same levels of available P during all the study period.

#### 3.3. Microbial biomass carbon and soil basal respiration

One of the parameters most directly affected by the fire in the soil was the  $C_{mic}$ , as can be observed in Fig. 8, very low values were registered in all the experimental areas during the first two soil samplings, these values being below 300 mg C kg<sup>-1</sup> soil. In general the average content of  $C_{mic}$  in forest soils of our region is in the range of 800–1200 mg C kg<sup>-1</sup> soil (Zornoza et al., 2007; Garcia-Orenes et al., 2012). It has been observed that the control area showed a good recovery of microbial populations after the third sampling reaching mean  $C_{mic}$  values of 1200 mg C kg<sup>-1</sup> at the end of the study period, while the average value in the soils of the SL treated area was below 600 mg C kg<sup>-1</sup> at the end of the study.

A similar behaviour has been observed in BSR with a lower activity immediately after fire in all the samples studied (Fig. 9). The soil of the control area was recovering microbial activity in the following samplings while the area with SL treatment showed lower values relative to the control, this being statistically significant at the two last soil sampling periods (Table 1).

The RDA performed on all data (Fig. 10) showed that the first two axes could explain 72% of the total variation. Axis 1 separated SL samples of C samples and explained 58%. All soil physical, chemical and microbial properties were significant (p < 0.05). The variable AS accounted for a large amount of the variation in the distribution of samples along axis 1. The RDA has showed an important and significant difference of behaviour in the majority of the properties studied between SL and C



Fig. 6. Temporal changes of nitrogen (N) from untreated-control (C) area versus salvage logging (SL) treated area during the 2 years of study. Different letters above the bars indicate significant differences between soil sampling periods (1–5), in capital and bold for SL treatment.



Fig. 7. Temporal changes of available phosphorous (P) from untreated-control (C) area versus salvage logging (SL) treated area during the 2 years of study. Different letters above the bars indicate significant differences between soil sampling periods (1–5), in capital and bold for SL treatment.

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Fig. 8. Temporal changes of microbial biomass carbon (C<sub>mic</sub>) (P) from untreated-control (C) area versus salvage logging (SL) treated area during the 2 years of study. Different letters above the bars indicate significant differences between soil sampling periods (1–5), in capital and bold for SL treatment.



Fig. 9. Temporal changes of basal soil respiration (BSR) (P) from untreated-control (C) area versus salvage logging (SL) treated area during the 2 years of study. Different letters above the bars indicate significant differences between soil sampling periods (1–5), in capital and bold for SL treatment.

samples, these differences have been specially marked in microbiological properties  $C_{mic}$  and BSR and N that were strongly related with samples from the control area.

#### 3.4. Plant recovery

No significant differences were observed in the number of plant species recorded (richness) comparing plots with SL treatment versus



**Fig. 10.** RDA analysis performed with all data. AS: aggregate stability; FC: field capacity; P: available phosphorous; OM: organic matter content; N: nitrogen; BSR: basal soil respiration, C<sub>mic</sub>: microbial biomass carbon.

control (untreated) plots (Table 2) in the three samples conducted, but the number of individuals of each species (evenness) was always significantly higher in C plots.

These differences were highly significant in the case of some species such as *Pinus halepensis*, that presented an average of 20 individuals in the SL plots and > 300 individuals in the C plots (in autumn 2014).

Regarding diversity results (Shannon-Weaver index) differences between treatments were not statistically significant (Table 2). In annex (supplement) are details of all plant species that appeared in both C and SL areas.

#### 4. Discussion

Post-fire management has engendered a great debate about the correct method to recover soil properties affected after fire (Beschta et al., 2004; Donato et al., 2006; Lindenmayer et al., 2004; McIver and Starr, 2001). In the last two decades SL has been a common practice after fire, but recent studies have reported that this treatment may impact ecosystem function and regeneration (Donato et al., 2006; Lindenmayer and Noss, 2006; Castro et al., 2010, 2011). In our study, post-fire SL management clearly produced a general degradation of soil as indicated by most of the soil parameters analysed. Physically, soil suffered compaction, indicated by an increase in bulk density and a loss of AS. The results of BD are similar to those observed by Wagenbrenner et al. (2015) in a study of the effect of SL on soil in the western USA; they found that the soil compaction due to logging using machinery extended to a depth of at least 10 cm and relatively few passes of the logging equipment resulted in substantial soil compaction. In our case we think it is more due to the loss of vegetation cover, the progressive loss of OM during the study period, and the destruction of a part of the aggregates producing an increase in BD. Soil OM content and AS are very closely and positively correlated in the forest soils of the region (Chrenková et al., 2014). The loss of OM, and the

Table 2

Plant recovery parameters measured and differences between treatments for every monitored period. Significant at: \*p < 0.05, \*\*p < 0.01; \*\*\*p < 0.001; ns: not significant (p > 0.05).

	Autumn 2013		Spring 2014			Autumn 2014			
	Richness	Evenness	H′	Richness	Evenness	H′	Richness	Evenness	H′
C SL	9.5 ± 1.2 8.7 ± 1.6 ns	$222.3 \pm 58.0 \\ 66.2 \pm 40.8 \\ *$	$\begin{array}{l} 0.55  \pm  0.06 \\ 0.64  \pm  0.05 \\ \mathrm{ns} \end{array}$	$\begin{array}{c} 12.0\pm2.0\\ 9.6\pm2.5\\ ns \end{array}$	$221.5 \pm 58.8 \\ 77.4 \pm 22.0 \\ *$	$\begin{array}{c} 0.64  \pm  0.10 \\ 0.72  \pm  0.03 \\ \mathrm{ns} \end{array}$	$\begin{array}{c} 10.2\pm0.9\\ 8.2\pm1.8\\ ns\end{array}$	$\begin{array}{c} 275.6  \pm  19.8 \\ 104.9  \pm  36.6 \\ {}^{**} \end{array}$	$\begin{array}{c} 0.58  \pm  0.04 \\ 0.51  \pm  0.05 \\ ns \end{array}$

effect on soil structure can also be responsible for the lower FC measured in the SL area in most of the soil samplings (Table 1). This alteration of soil structure produces an increase in the erosion rates, also a consequence of the lower vegetation cover (Larsen et al., 2009; Wagenbrenner and Robichaud, 2014). In the case of WR, soils of both treatments showed wettable conditions during the whole study period. This result is not strange since fires do not always induce or increase WR, because many factors are involved, such as temperatures reached during burning (Arcenegui et al., 2007), and also soil type and properties can control its presence and development by burning (Mataix-Solera et al., 2014).

The OM in the soils with post-fire SL treatment decreased from 7% to <3% in first 5 cm soil depth; this loss of OM is higher than losses observed as a direct consequence of high intensity forest fires in the Mediterranean area (Mataix-Solera et al., 2002). However in the case of the control area the OM content remained stable without decreasing in the whole study period. Some authors suggest that the decrease in OM content after the fire can be due to erosion (Hatten et al., 2005). In our case, the results of the SL area suggest this area has suffered higher erosion rates than the C area, and this fact could be the main cause of the progressive reduction of OM content together with conditions more prone to OM mineralization rates, this means, less vegetation cover and higher soil temperatures.

Microbiological parameters respond very quickly to perturbations in soil, and in this study we observed that both fire and post-fire management have a direct impact on the soil microbial biomass and activity. The effect of fire on microbial biomass (C<sub>mic</sub>) could be observed in the first two soil samplings with very low values of this parameter. This is as a consequence of the heat impact on soil microbiology (Mataix-Solera et al., 2009). SL treatment also affected C<sub>mic</sub> since the recovery with time was much lower in SL compared to C area. Microbial activity measured as BSR also showed lower values at the beginning of the study with an increasing trend for the control and a decreasing trend in the SL treated area. Thus both parameters have been consistently affected by the post-fire SL treatment. Keeping the burnt wood over the soil surface avoided the loss of OM content and may supply nutrients to the soil that have encouraged the microbial population and its activity (Harmon et al., 1986; Grove, 2003; Coleman et al., 2004), our study has reported higher levels of OM, N and P in the soil of the control area without post-fire treatment. Also the trees felled on the surface would facilitate wood-soil contact and decomposition (Maser and Trappe, 1984; Harmon et al., 1986) explaining a higher BSR in these soils compared with soil without wood. The maintaining the burnt wood on the soil could also help to reduce the soil desiccation produced by the higher soil heating expected over bare soil or with less vegetation cover (Stoddard et al., 2008; Castro et al., 2011). Burnt trees and branches can act as nurse structures improving microclimatic conditions for plant regeneration (Harmon et al., 1986; Lindenmayer and Noss, 2006; Stoddard et al., 2008). These conditions promoted microorganism development and increased the rate of respiration (Marañón-Jiménez et al., 2011). On the other hand the post-fire treatment increased the negative effect of fire in the soil producing degradation processes as showed by the slower and lesser development of soil microbial populations that are closely related with structure, soil OM content and other properties (García-Orenes et al., 2010). Some other studies showed that these kinds of treatments could affect soil microbial communities (Jennings et al., 2012) and nutrient cycling (Brais et al., 2000).

In addition, SL may damage the bank of seedlings and affect plant regeneration after fire, reducing plant density (Martínez-Sánchez et al., 1999; McIver and Starr, 2001), as has been observed in our study. The significantly greater abundance of individuals in the C plots means a greater plant cover. This fact represents better soil protection in the medium and long-term. This data indicates that the SL treatment clearly promoted a significant decrease in the soil seed bank that would result in a slower recovery of the original vegetation. Our study is in agreement with some other authors who observed that post-fire SL treatment reduces the vegetation cover (Serrano-Ortiz et al., 2011; Wagenbrenner et al., 2016).

#### 5. Conclusions

After two years of research, results showed significant soil degradation as a consequence of the salvage logging treatment. Most of the soil parameters studied showed differences between the treatments, soil OM content in first 5 cm of topsoil being less than half in SL plots in comparison with C area. BSR, C<sub>mic</sub> and AS were also statistically significant lower in SL plots. BD increased as a consequence of the SL treatment. In conclusion, we can affirm that with this type of soil, which is very vulnerable to soil degradation by erosion, the SL treatment has a very negative effect on the ecosystem, which was also reflected in the evenness of plant species. In many cases the reason given for post fire cuttings is because potential epidemic risk in unburned near forest. This only has justification in the borders between burned and unburned, where some trees are damaged but still alive. In the case that forest managers recommend to remove the post-fire vegetation we suggest to wait more time till soil is more protected by herbs and not bare and vulnerable to erosion, or doing in combination with some protective treatment like mulching. More research is needed in order to identify the SL effects in different soil type conditions, at different times after fire, and/or in combination with soil conservation treatments like mulching.

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# The impact of post-fire salvage logging on microbial nitrogen cyclers in Mediterranean forest soil



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#### HIGHLIGHTS

## G R A P H I C A L A B S T R A C T

- Salvage logging post-fire affects the properties of Mediterranean forest soil.
- Tree retention post-fire increases pools of *nifH*, *amoA*-B, *amoA*-Arch, *nirK* & *nosZ*.
- OM, avP, N & aggregate stability impact N cycler abundance in forest soil postfire.
- Microaggregates are hotspots for N cyclers particularly under salvage logging.
- Greater N cycling can support post-fire re-vegetation improving ecosystem recovery.



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#### ABSTRACT

Forest fires are a regular occurrence in the Mediterranean basin. High severity fires and post-fire management can affect biological, chemical and physical properties of soil, including the composition and abundance of soil microbial communities. Salvage logging is a post-fire management strategy, which involves the removal of burnt wood from land after a fire. The main objective of this work was to evaluate the impact of post-fire salvage logging and microaggregation on soil microbial communities, specifically on the abundance of nitrogen cyclers and, thus, the potential of the soil for microbial nitrogen cycling. The abundance of nitrogen cyclers was assessed by quantification of microbial nitrogen cycling genes in soil DNA, including *nifH* (involved in nitrogen fixation), nirS/K and nosZ (involved in denitrification), amoA-B and amoA-Arch (involved in bacterial and archaeal nitrification, respectively). It was demonstrated that salvage logging reduced bacterial load post-fire when compared to tree retention control and resulted in significant changes to the abundance of functional bacteria involved in nitrogen cycling. Microbial gene pools involved in various stages of the nitrogen cycle were larger in control soil than in soil subjected to post-fire salvage logging and were significantly correlated with organic matter, available phosphorous, nitrogen and aggregate stability. The microaggregate fraction of the soil, which has been associated with greater organic carbon, was shown to be a hotspot for nitrogen cyclers particularly under salvage logging. The impact of post-fire management strategies on soil microbial communities needs to be considered in relation to maintaining ecosystem productivity, resilience and potential impact on climate change.

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#### 1. Introduction

Fires have been a common natural disturbance from late Devonian Period (Schmidt and Noack, 2000). Over the last six decades human

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activity, mainly land use change such as agricultural abandonment, has influenced the pattern, frequency and intensity of fire in the Mediterranean basin (Cerdà and Mataix-Solera, 2009; Pausas and Keeley, 2009). Most currently forested areas in the Mediterranean were previously cultivated but have been abandoned for the last 30-50 years. The lack of management during this transition period from cropping to forest recolonization has led to an accumulation of fuel, resulting in severe fires in some areas (Mataix-Solera and Cerdà, 2009). High severity fires modify soil properties, mainly depending on the peak temperatures, the duration of the fire, soil type and pre-fire conditions (Certini, 2005). Fires affect many biological, chemical and physical properties of soil (Ballard, 2000; Jiménez Esquilin et al., 2008; Yeager et al., 2005), with soil microorganisms being affected even by relatively low temperatures (Neary et al., 1999; Bárcenas-Moreno and Bååth, 2009). Soil microbial communities are also sensitive to changes in other soil properties caused by fire, such as the increase in soil pH from ash accumulation (Mataix-Solera et al., 2009), and volatilization of nitrogen (N) at temperatures around 200 °C (Chandler et al., 1983). Despite a loss of total N by combustion, inorganic N tends to increase as a result of mineralization caused by fire (Rasion et al., 1985; Carballas et al., 1993). This increase however can be very ephemeral because  $NO_3^-$  can be easily leached through the soil profile.

Certain post-fire management strategies can have a negative impact on soils, an impact that is potentially more damaging than the fire itself (Mataix-Solera et al., 2015). Salvage logging (SL) is a post-fire management strategy that involves the extraction of burnt wood using heavy machinery, cutting the trunks, and dragging them over the soil surface. Studies on the vegetation dynamic in areas where post-fire SL has been carried out concluded that it had a negative impact on plant recovery (e.g.: González-Ochoa et al., 2003; Martínez-Sánchez et al., 1998; Pausas et al., 2004). However, there is little information on the impact of post-fire SL on soil properties, especially in regards to soil microbial communities. Marañón-Jiménez et al. (2011) observed a decrease in soil respiration after post-fire SL. García-Orenes et al. (2017), observed significant soil degradation in Sierra de Mariola Natural Park, Alcoi, Alicante (E Spain) two years after post-fire SL treatment, which affected the recovery of soil physical, chemical and microbial parameters compared with control areas where burnt trees were retained. Differences between SL treatment and tree-retention controls included reduced soil organic matter (OM) content in topsoil (top 5 cm) to <50%, lower basal soil respiration (BSR), decreased microbial biomass carbon (MBC) and lower aggregate stability (AS). Soil bulk density (BD) increased as a consequence of SL. This was also reflected in the lower density and evenness of vegetation (García-Orenes et al., 2017).

Many soil functions are carried out by soil microbial communities, however, there is little information available on the recovery of microbial functional groups following fires. N cycling is one of most important soil functions carried out by microorganism (Fitter et al., 2005; Wallenstein and Vilgalys, 2005; He et al., 2007). N fixing bacteria reduce  $N_2$  to  $NH_4^+$ , producing most of the available N in the biosphere (Brankatschk et al., 2011; Mackelprang et al., 2011). Other microorganisms decompose organic N into NH<sub>4</sub><sup>+</sup> (Zhou et al., 2012) and nitrifying microorganisms oxidize  $NH_4^+$  to  $NO_2^-$  and then  $NO_3^-$ , the preferred N form for plants (Horz et al., 2004; Fierer et al., 2012). Denitrifying microorganism reduce NO<sub>3</sub><sup>-</sup> to NO, N<sub>2</sub>O and N<sub>2</sub>, returning N to atmosphere and completing the N cycle (Braker et al., 1998; Houlton and Bai, 2009). Therefore, beyond its nutritional value, N cycling is also important in a number of other environmental contexts, for example in controlling the nitrous oxide emission. Our study is adding knowledge on the effects of post-fire management, in particular salvage logging, on N-cycling microbes in forest soils.

The main objectives of this work were to evaluate the impact of post-fire SL on the potential of the soil for microbial N cycling, and relate the soil capacity for N cycling with other parameters of soil health, such as OM, AS, MBC and BSR. Soil potential for N cycling was studied by quantification of genes involved in N cycling in total soil DNA, including *nifH* (involved in N fixation), *nirS/K* and *nosZ* (involved in denitrification) as well as *amoA*-B and *amoA*-Arch (involved in bacterial and archaeal nitrification, respectively). Microbial diversity has recently been shown to be highest in the micro-aggregate fraction of soil (Rabbi et al., 2016), therefore, N cycling potential in the top soil was compared to that in the microaggregate fraction (sieved between 63 and 250 µm). The retention of OM in undisturbed soil, such as under no-tillage cropping systems, stabilizes microaggregates and thus reduces the turnover of macroaggregates (Six et al., 2000). We hypothesize that microaggregates in burnt forest soil subjected to SL, which contains less organic carbon and reduced aggregate stability (García-Orenes et al., 2017), will show decreased microbial N cycling when compared to control areas where trees were retained.

#### 2. Material and methods

#### 2.1. Study site

The study area is located in "Sierra de Mariola Natural Park" in Alcoi, Alicante (E Spain, coordinates: 38°43′59″N, 0°29′16″W). The climate is Mediterranean, characterised by a dry-hot summer and a wet-warm spring, autumn and winter. This area often suffers 3-4 month summer droughts, usually from late June to September. The annual average precipitation is 490 mm, which falls mainly in October-November (maximum rainfall in October, 71 mm). The mean monthly temperature is 14.8 °C (summer: 22.7 °C, winter: 7.9 °C). The forest is composed mainly of Pinus halepensis trees, around 40 years of age, with an understory of typical Mediterranean shrubs species, for example Quercus coccifera, Rosmarinus officinalis, Thymus vulgaris and Brachypodium retusum. The soil is classified as a Typic Xerorthent (Soil Survey Staff, 2014) developed over marls with a low deep, and is very vulnerable to erosion and degradation processes with a loam soil texture with 45, 39 and 17% of sand, silt and clay, respectively, and 44% of carbonates (García-Orenes et al., 2017).

A forest fire of moderate severity, as defined by Keeley (2009) according to the loss of organic matter above- and belowground, occurred in July 2012 affecting a total of 546 ha. Six months after the forest fire, in February 2013, salvage logging (SL) was carried out in a part of the affected forest. SL consisted of complete extraction of the burned wood using heavy machinery.

#### 2.2. Experimental design and soil sampling

Three plots of 4 m<sup>2</sup> were set up in the burnt forest area where postfire SL had been carried out (SL) and another three plots in a similarly burnt area nearby where trees were retained, which was used as untreated control (C). In May 2014 (22 months post fire and 15 months after SL treatment) three soil samples (up to 5 cm depth) were collected from the A horizon mineral soil of each plot (n = 9 samples per treatment, SL and C), sieved at 2 mm and kept at 4 °C for soil property analyses, and a portion frozen at -20 °C for DNA extraction. A fresh subsample of each sample was sieved through a mesh (63–250 µm) to obtain microaggregates (9 samples per treatment, as above) and a portion was kept at -20 °C for DNA extractions. The samples were denoted: SL-intact core (SL-I), SL-microaggregates (SL-M), C-intact core (C-I) and C-microaggregates (C-M). The properties of the SL and C soils were determined by García-Orenes et al. (2017) and are presented in Table 1.

#### 2.3. DNA extraction and quantitative PCR analysis

DNA was extracted from 0.25 g of each soil sample: SL-I, C-I, SL-M and C-M (nine samples per treatment: three replicate plots and three samples per plot). Total soil DNA extraction was performed using the DNA PowerSoil kit (Mo Bio, Carlsbad, USA) with the following modifications to the manufacturer's instructions: initial vortexing was at 300 rpm for 20 min (STD 3500 Shaker VWR) and DNA was eluted

#### Table 1

Soil physical-chemical, biological and biochemical properties and plant recovery parameters in May 2014 (García-Orenes et al., 2017).

	Salvage logging	Control <sup>a</sup>
Aggregate stability (%)	$71.34\pm6.04a$	$84.61\pm3.93b$
Nitrogen Kjeldahl (%)	$0.11\pm0.01a$	$0.26\pm0.05b$
Available phosphorus (g kg <sup>-1</sup> )	$5.94 \pm 2.23a$	$36.5 \pm 16.4b$
Soil organic matter content (%)	$2.62\pm0.8a$	$6.61 \pm 1.62b$
Field capacity (%)	$89.6\pm6.9a$	$105.2 \pm 9.7b$
Basal soil respiration (C-CO <sub>2</sub> ( $\mu$ g h <sup>-1</sup> g <sup>-1</sup> soil))	$1.42\pm0.37a$	$2.31\pm0.48a$
Microbial biomass carbon (mg C kg <sup>-1</sup> soil)	$574\pm72a$	$1040 \pm 81b$
Plant richness	$9.6\pm2.5a$	$12.0\pm2.0a$
Plant evenness	$77.4 \pm 22.0a$	$221.5\pm58.8b$
Plant Shannon index H'	$0.64\pm0.10\text{a}$	$0.72\pm0.03a$

Soil samples were from 0 to 5 cm depth. Values in rows sharing the same letter do not differ significantly (one-way ANOVA, P < 0.05, n = 9).

<sup>a</sup> Control = tree retention

with 65  $\mu$ L elution buffer. DNA yield and purity was measured using a nanodrop (ND-1000 spectrophotometer, NanoDrop Technologies) and the quality of the total DNA (expecting intact DNA of high molecular weight) was further assessed by agarose gel electrophoresis.

Quantitative PCR (gPCR) analyses were used to quantify the total bacterial 16S rRNA gene as well as the N cycling genes in each soil DNA sample. Each sample was assayed in triplicate (three technical replicates of nine independent replicate = 27 per treatment) on a CFX96 Touch Real-Time PCR detection system (Bio-Rad laboratories, CA, USA). Each 25  $\mu$ L qPCR reaction contained 1 $\times$  iQ SYBR Green Supermix (Bio-Rad Laboratories, CA, USA), 400 nM each forward and reverse primers, 2.5 µg/µL ultrapure BSA (Invitrogen, CA, USA), 0.5 µL PCR grade DMSO (Sigma-Aldrich, MO, USA), 1 µL of DNA template and RNase/DNase-free water. N cycling genes amplified included 1) nirS and nirK and nosZ, encoding nitrite reductases and nitrous oxide reductase involved in denitrification, 2) nifH, encoding the nitrogenase of diazotrophs and 3) archaeal and bacterial amoA-Arch and amoA-B, respectively, encoding ammonia monooxygenase in nitrifiers (ammonium-oxidizing microbes). The primer sets used and the qPCR cycling conditions for each primer set are shown in Table 2.

PCR products amplified from soil DNA were cloned into pGEM-T using a pGEM-T Easy Vector System II (Promega, WI, USA) to produce standards. Successful cloning and transformation of the target genes was confirmed by sequencing. In all qPCR assays, all samples were amplified in parallel with a triplicate serial dilution  $(10^1-10^7$  gene copies per reaction) of these standards. The efficiencies of qPCR assays were determined by amplification of a serial dilution of soil DNA (5 fold dilution series, from 5 to 0.008 µL DNA per reaction), to give standard curves with efficiencies ranging from 68.9 to 88.7%, with R2 values ranging from 0.988 to 0.997. To assess presence of inhibitors of amplification such as humic and fulvic soil contaminants, the efficiencies and standard curves from amplification of serial dilution of soil DNA were compared to amplification curves and efficiencies from standard plasmids. Soil DNA and standards showed similar efficiencies and standard curve slopes, with no inhibition detected.

Duplicate no-template controls were run for each qPCR assay, which gave null or negligible values. Melt curve analysis and gel electrophoresis were used to confirm amplicon specificity (Data not shown). Gene copy abundance was normalized to gram of dry soil to give results on a biologically significant scale, assuming similar DNA isolation efficiency across samples. Such assumption is appropriate in this study since relative (vs. absolute) quantification was measured.

#### 2.4. Statistical analysis

The adjustment of the data to a normal distribution for all properties analysed was verified with the Kolmogorov-Smirnov test at P < 0.05. Measured variables were submitted to a one-way ANOVA, assuming equal variance and the separation of means was carried out according

Table	2		
- ·			

Target gene	Primers	Reference
nirS	Cd3aF: GTSAACGTSAAGGARACSGG	Throbäck et al. (2004)
	R3cd: GASTTCGGRTGSGTCTTGA	
nirK	nirk876: ATYGGCGGVCAYGGCGA	Henry et al. (2004)
	nirk1040: GCCTCGATCAGRTTRTGGTT	
nosZ	nosz2F:	Henry et al. (2006)
	CGCRACGGCAASAAGGTSMSSGT	
	nosz2R: CAKRTGCAKSGCRTGGCAGAA	
nifH	MMF2: TNATCACCKCNATCACTTCC	Pereg and McMillan,
	MMR1: CGCCGGACKWGACGATGTAG	unpublished <sup>a,b</sup>
атоА-В	amoa1F: GGGGTTTCTACTGGTGGT	Rotthauwe et al. (1997)
	amoa2R: CCCCTCKGSAAAGCCTTCTTC	
amoA-arch	Arch-AmoAF:	Francis et al. (2005)
	STAATGGTCTGGCTTAGACG	
	Arch-AmoAR:	
	GCGGCCATCCATCTGTATGT	
16S rRNA	16 s Forward:	Mohammadi et al. (2003)
gene	TCCTACGGGAGGCAGCAGT	
	16 s reverse:	
	GGACTACCAGGGTATCTAATCCTGTT	

Fragments amplified with these set of primers from a variety of soils were cloned into plasmids and the correct size fragments were amplified from 30 transformed *E. coli* colonies for each gene. The fragments cloned were verified as diverse *nifH* sequences.

<sup>a</sup> Based on multiple sequence alignment of available *nifH* sequences from GenBank database to identify conserved regions, and degenerate primers within these regions designed using Primer3 (http://frodo.wi.mit.edu/).

<sup>b</sup> The qPCR *nifH* primers used in this study were designed to overcome issues with previously published primer sets that yielded non-specific products. The qPCR cycle used for these primers is: 95 °C 10 mins; 40 cycles of 15 s 95 °C, 30 s 60 °C, 30 s 72 °C.

to the average post hoc Tukey test with differences considered significant at P < 0.05. Pearson's correlation coefficients (R) were calculated to quantify the linear relationship between parameters.

The relationship between the abundance (copy number  $g^{-1}$  soil) of the various genes as well as between gene abundance and soil properties determined by García-Orenes et al. (2017) (Table 1) were analysed by principal component analysis (PCA) with Varimax normalized rotation in order to determine the influence of the post-fire management strategies on the abundance of the N cycling genes. All the results were subjected to a correlation analysis with soil parameters measured using Pearson's rank correlation coefficients. SPSS software (Statistical Program for the Social Sciences 23.0) was used for all statistical analysis.

#### 3. Results

Figs. 1 to 4 show the concentration of total soil DNA, as well as the abundance of the prokaryotic 16S rRNA gene and the six N-cycle genes studied, in intact soil and in the microaggregate fractions of C and SL soil samples.

The average total soil DNA concentrations in SL-I soil samples (9.0  $\pm$  1.6 µg DNA g<sup>-1</sup> soil) and SL-M (13.8  $\pm$  4.9 µg DNA g<sup>-1</sup> soil) were 35% lower than in C soil corresponding samples (14.0  $\pm$  2.0 for C-I and 21.0  $\pm$  5.7 µg DNA g<sup>-1</sup> soil for C-M) (Fig. 1A). Microaggregates had 35% higher concentrations of DNA than intact soil in both SL and C soils (Fig. 1A). These differences in total DNA concentrations were statistically significant. The 16S rRNA gene abundance was also significant. In the SL soil (3.4E  $\pm$  07 and 4.5E  $\pm$  07 gene copy number g<sup>-1</sup> soil for SL-I and SL-M, respectively) than in the C soil (1.4E  $\pm$  08 and 1.7E  $\pm$  08 gene copy number g<sup>-1</sup> soil for C-I and C-M, respectively), however, there was no significant difference between 16S rRNA abundance in intact soil and the microaggregate fraction in either the SL or C soils (Fig. 1B).

The abundance of the *nifH* gene, encoding the nitrogenase enzyme in N fixing bacteria, was three fold higher in the intact C-I soil (2.7E + 06 gene copy number  $g^{-1}$  soil) than in the intact SL-I soil (1.0E + 06 gene copy number  $g^{-1}$  soil) (Fig. 2). However, similar *nifH* copy numbers were found in the microaggregates of SL-M and C-M (2.9E +



**Fig. 1.** Abundance of soil total DNA (A) and 16S rRNA gene (B) (mean  $\pm$  standard deviation) in soils under different treatments analysed by one-way ANOVA. SL-I: soil under salvage logging treatment sieved at 2 mm; SL-M: soil under salvage logging treatment sieved between 63 and 250  $\mu$ m; C-I: control soil with tree retention sieved at 2 mm; C-I: control soil with tree retention sieved at 2 mm; C-I: control soil with tree retention sieved between 63 and 250  $\mu$ m; C-I: control soil with tree retention sieved at 2 mm; C-I: control soil with tree retention sieved between 63 and 250  $\mu$ m; C-I: control soil with tree retention sieved between 63 and 250  $\mu$ m; C-I: control soil with tree retention sieved between 63 and 250  $\mu$ m; C-I: control soil with tree retention sieved between 63 and 250  $\mu$ m; C-I: control soil with tree retention sieved between 63 and 250  $\mu$ m; C-I: control soil with tree retention sieved between 63 and 250  $\mu$ m; C-I: control soil with tree retention sieved between 63 and 250  $\mu$ m; C-I: control soil with tree retention sieved between 63 and 250  $\mu$ m; C-I: control soil with tree retention sieved between 63 and 250  $\mu$ m; C-I: control soil with tree retention sieved between 63 and 250  $\mu$ m; C-I: control soil with tree retention sieved between 63 and 250  $\mu$ m; C-I: control soil with tree retention sieved between 63 and 250  $\mu$ m; C-I: control soil with tree retention sieved between 63 and 250  $\mu$ m; C-I: control soil with tree retention sieved between 63 and 250  $\mu$ m; C-I: control soil with tree retention sieved between 63 and 250  $\mu$ m; C-I: control soil with tree retention sieved between 63 and 250  $\mu$ m; C-I: control soil with tree retention sieved between 63  $\mu$ m; C-I: control soil with tree retention sieved between 63  $\mu$ m; C-I: control soil with tree retention sieved between 63  $\mu$ m; C-I: control soil with tree retention sieved between 63  $\mu$ m; C-I: control soil with tree retention sieved between 63  $\mu$ m; C-I: control soil with tree retention sieved between 63  $\mu$ m; C-I: control soil with tree re

06 and 3.3E + 06 gene copy number  $g^{-1}$  soil, respectively) (Fig. 2). While there was a higher abundance of *nifH* in SL-M than in SL-I, the gene abundance in C-M was similar to that in C-I (Fig. 2).

Quantification of genes related to denitrification indicated that nosZ was present at higher copy numbers in SL-M (1.6E + 06 gene copy number  $g^{-1}$  soil) than in SL-I (6.2E + 05 gene copy number  $g^{-1}$  soil) and higher in C-M (3.2E + 06 gene copy number  $g^{-1}$  soil) than in C-I  $(1.9E + 06 \text{ gene copy number } g^{-1} \text{ soil})$  (Fig. 3A). nosZ was also more abundant in C-I than in SL-I, and in C-M than in SL-M. nirK was more abundant in the C-I soil  $(1.7E + 07 \text{ gene copy number g}^{-1} \text{ soil})$  than in the SL-I soil (8.7E + 06 gene copy number  $g^{-1}$  soil) and was also present at greater abundance in the C-M (1.4E + 07 gene copy)number  $g^{-1}$  soil) than the SL-M (5.7E + 06 gene copy number  $g^{-1}$  soil) (Fig. 3B). However, there were no significant differences in copy number between C-I and C-M, or between SL-I and SL-M (Fig. 3B). There were no significant difference in *nirS* copy numbers between any of the samples (Fig. 3C). Copy number per gram of soil were similar in magnitude for *nirK* and *nirS*, and both were approximately a level of magnitude higher than copy number of *nosZ* (Fig. 3).

The distribution of the archaeal and bacterial nitrification *amoA* genes showed similar patterns (Fig. 4). Copy numbers of the *amoA*-Arch (Fig. 4A) and the bacterial *amoA*-B (Fig. 4B) were both higher in C-I (6.4E + 04 and 4.9E + 04 gene copy number  $g^{-1}$  soil, respectively) than in SL-I (1E + 04 and 4.1E + 03 gene copy number  $g^{-1}$  soil, respectively), as well as higher in C-M (9.7E + 04 and 9.3E + 04 gene copy number  $g^{-1}$  soil, respectively) than in SL-M (2.7E + 04 and 2.1E + 04 gene copy number  $g^{-1}$  soil, respectively) than in SL-M (2.7E + 04 and 2.1E + 04 gene copy number  $g^{-1}$  soil, respectively) (Fig. 4). Both genes were also more abundant in C-M than in C-I as well as more abundant in SL-M that in SL-I (Fig. 4). Interestingly, the abundance of the archaeal and the bacterial *amoA* genes had a similar level of magnitude under all treatments (Fig. 4).

Soil properties and plant richness and evenness data determined by García-Orenes et al. (2017) were used to statistically analyse whether there is significant correlation between these factors and the abundance of 16S rRNA gene as well as genes related to the N cycle. Since the previous study only analysed the intact soil (García-Orenes et al., 2017) we used only the data from the gene abundance in intact soil SL-I and C-I in these correlation analyses. A significant positive correlation was previously found between soil OM, N, AP, AS, MBC and BSR (García-Orenes et al., 2017). While total DNA concentration significantly correlated with the soil properties, it only positively correlated with abundance of three genes: nifH, nosZ, nirS, in addition to the 16S rRNA gene (Table 3). All of the soil properties other than MBC were also positively correlated with the abundance of *nifH* (Table 3). The abundance of the 16S rRNA gene, which is present in different bacterial and archaeal species, was positively and significantly correlated with all of the soil properties studied as well as with the abundance of *nirS*, but not significantly correlated with the other genes (Table 3). The abundance of nosZ was correlated with soil OM, AP, AS and BSR and the abundance of amoA-B with MBC while the abundance of nirS, nirK, amoA-Arch and did not show significant correlation with the soil properties (Table 3).

Correlation analysis on the copy numbers of different genes involved in the N cycle showed that the abundance of soil *nosZ*, *nirS* and *amoA*-Arch were significantly correlated (Table 3). The abundance of *nifH* was correlated to that of *nosZ*, but *nirK* and *amoA*-B showed no significant correlation with any of the other N cycling genes (Table 3).

PCA was performed to evaluate the influence of the post-fire management strategy on the abundance of the 16S rRNA gene and N cycling genes in both intact soil and in the microaggregate fraction of soils (Fig. 5). The first two components explained 90% of the total variation with eigenvalues >1.0. The first component PC1 explained 70% of the variation and separated C from SL, with *nirS*, *nifH* and *amoA*-Arch having



**Fig. 2.** Abundance of *nifH* gene (mean ± standard deviation) in soil with different treatment after one-way ANOVA. SL-I: soil under salvage logging treatment sieved at 2 mm; SL-M: soil under salvage logging treatment sieved between 63 and 250 μm; C-I: control soil with tree retention sieved at 2 mm; C-I: control s



Fig. 3. Abundance of *nosZ* gene (A), *nirK* gene (B) and *nirS* gene (C) (mean ± standard deviation) in soil with different treatment after one-way ANOVA. SL-I: soil under salvage logging treatment sieved at 2 mm; SL-M: soil under salvage logging treatment sieved between 63 and 250 µm; C-I: control soil with tree retention sieved at 2 mm; C-I: control soil with tree retention sieved at 2 mm; C-I: control soil with tree retention sieved at 2 mm; C-I: control soil with tree retention sieved at 2 mm; C-I: control soil with tree retention sieved between 63 and 250 µm; C-I: control soil with tree retention sieved at 2 mm; C-I: control soil with tree retention sieved at 2 mm; C-I: control soil with tree retention sieved between 63 and 250 µm.

loading values of 0.93, 0.91 and 0.89, respectively. The second component PC2 explains 20% of the variance and separated the intact soil of each treatment from its microaggregate fraction with *nosZ* and *16S r*RNA gene having loading values of 0.70 and 0.68, respectively.

Fig. 6 shows the result of a PCA, which included all of the soil properties studied (Table 1), total DNA and the abundance of 16S rRNA gene and genes involved in N cycling from intact soil samples. The two first components have explained 88.9% of the variance with eigenvalues >1.0. The first component PC1 explained 79.7% of the variation and separated Soil C from Soil SL, with OM, N, AS, MBC and BSR having loading values of 0.96, 0.95, 0.93, 0.85 and 0.79, respectively.

#### 4. Discussion

Our study enhances the knowledge on the effect of post-fire forest management on the abundance of soil bacteria, in particular those involved in N cycling. Our results show a decline in abundance of the 16S rRNA gene after salvage logging, suggesting that, while tree retention allows the recovery of soil bacterial communities in burnt forest soil, SL reduces the ecosystem resilience and recovery of bacterial communities. This is in agreement with previous finding of Dahlberg et al. (2001) and Twieg et al. (2007), who found a decline in the abundance of microorganism after salvage logging, and Holden et al. (2016), who demonstrated that forest clearing has a negative impact on soil microbes. Tree management by partial harvesting appears to have less impact on microbial biomass in soils with low pH and high carbon content (Gömöryová et al., 2017). García-Orenes et al. (2017) found that SL resulted in slower recovery of MBC and BSR in Sierra de Mariola. High correlations of OM content, N and available P with bacterial abundance in neutral soil (pH approx. 8.0 for all samples in C and SL) of Sierra de Mariola, suggest that these soil parameters could explain the lower abundance of bacterial 16S rRNA gene under SL. Nevertheless, other soil properties, including physical parameters, such as soil compaction, should not be ignored. Timber harvesting using heavy machinery has been shown to have a significant impact on soil microbial communities in Northern coniferous forests (Hartmann et al., 2012) and controlled fundamental experiments showed that forest soil compaction was associated with a persistent change in soil microbiota, with sandy soils being more resistant to the effects of compaction than clayey soils (Hartmann et al., 2014). Indeed, the bulk density of the relatively sandy soil (45%) of Sierra de Mariola measured in May 2014 was higher under SL (0.8 + $0.05 \text{ g cm}^{3-1}$ ) than under control (C) conditions  $(0.72 + 0.05 \text{ g cm}^{3-1})$ (García-Orenes et al., 2017), suggesting that compaction in addition to tree removal could possibly be the cause of the decrease in soil bacterial abundance in SL soil. Compaction affects moisture and oxygen levels in the soil, with anaerobic bacteria being significantly associated with compacted forest soils (Hartmann et al., 2014).

Our results clearly demonstrate the importance of microaggregation, in particular in the disturbed SL soil. Rabbi et al. (2016) observed higher diversity of bacteria in soil microaggregates than in macroaggregates,



Fig. 4. Abundance of *amoA*-Arch gene (A) and *amoA*-B (B) (mean ± standard deviation) in soil with different treatment after one-way ANOVA. SL-I: soil under salvage logging treatment sieved at 2 mm; SL-M: soil under salvage logging treatment sieved between 63 and 250 µm; C-I: control soil with tree retention sieved at 2 mm; C-I: control soil with tree retention sieved between 63 and 250 µm.

Table 3

Correlation coefficients (r va	ues) for relationsh	ips between the different	physico-chemical soil	properties determined and	genes of soil (	n = 18)
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Parameter <sup>a</sup>	OM	Ν	AP	AS	MBC	BSR	nifH	nosZ	nirS	nirK	amoA-Arch	атоА-В	16S rDNA	Total DNA
OM	1	0.95**	0.91**	0.77**	0.75**	0.78**	0.70**	0.47*	ns	ns	ns	ns	0.76**	0.84**
Ν		1	0.88**	0.81**	0.80**	0.70**	0.68**	ns	ns	ns	ns	ns	0.79*	0.83**
AP			1	0.78**	0.66**	0.75**	$0.74^{**}$	$0.49^{*}$	ns	ns	ns	ns	0.63**	0.70**
AS				1	0.69**	$0.54^{*}$	0.64**	$0.56^{*}$	ns	ns	ns	ns	0.76**	0.63**
MBC					1	0.51*	ns	ns	ns	ns	ns	0.62**	0.72**	0.59**
BSR						1	0.53*	0.66**	ns	ns	ns	ns	0.53*	0.71**
nifH							1	0.67**	ns	ns	ns	ns	ns	0.67**
nosZ								1	ns	ns	0.56*	ns	ns	0.59*
nirS									1	ns	0.56*	ns	0.65**	0.66**
nirK										1	ns	ns	ns	ns
amoA-Arch											1	ns	ns	ns
атоА-В												1	ns	ns
16S rDNA													1	0.77**
Total DNA														1

ns: not significant. 16S rDNA: 16S rRNA gene. The results were confirmed using a non-linear method (Spearman) and similar values were obtained.

<sup>a</sup> OM: soil organic matter; N: Kjeldahl nitrogen; AP: available phosphorus; AS: aggregates stability; MBC: microbial biomass carbon; BSR: basal soil respiration.

\* Significant at P < 0.05

\*\* Significant at P < 0.01.

which was highly correlated with organic carbon in pasture, crop and woodland soils. The significant correlation between 16S rRNA gene abundance, OM and AS, suggests that the combination of OM and higher degree of aggregation in Soil C (control soil) support greater bacterial abundance than in forest soil that has undergone SL.

The quantification and characterization of N cycling communities, including N-fixation, denitrification and nitrification, can help assess ecosystem functioning in natural and agricultural systems (Kowalchuk and Stephen, 2001; Philippot, 2002), and provide information for predicting and mitigating greenhouse gas emissions (reviewed by Levy-Booth et al., 2014). Burning and logging can affect the concentrations of OM and N in soils (Grogan et al., 2000; Neary et al., 1999; Wan et al., 2001; García-Orenes et al., 2017) and the N-cycling microbial communities (Reich et al., 2001; Shaffer et al., 2000; Walley et al., 1996). The higher abundance of bacteria and archaea, especially those involved in N cycling, in control soil without post-fire tree removal than after SL is especially important in burned soil since improved N cycling could increase N pools available for re-vegetation following fire. This is in agreement with the higher plant richness and evenness in control soil than in the soil following post-fire SL.



**Fig. 5.** Principal component analysis performed on the abundance of different soil N cycling genes studied (*nifH*, *nirS*, *niK*, *nosZ*, *amoA*-Arch and *amoA*-B) and 16 s rRNA gene. SL-I: soil under salvage logging treatment sieved at 2 mm; SL-M: soil under salvage logging treatment sieved at 2 mm; C-I: control soil with tree retention sieved at 2 mm; C-I: control soil with tree retention sieved at 2 mm; C-I: control soil with tree retention sieved between 63 and 250 µm. Different letters above the bars indicate significant differences.

4.1. Effect of post-fire treatment on nitrogen fixation genes abundance in soil

Fire and logging both have long-term impacts on N-cycling bacteria (Goodale and Aber, 2001; Kennedy and Egger, 2010). In agreement with Shaffer et al. (2000), who concluded that logging removes a unique *nifH* gene pool from the soil in Douglas-fir forests, probably as a consequence of loss of organic material, we have shown that SL has decreased the abundance of *nifH* in fire affected soil when compared to control areas without tree removal.

Similarly to Kennedy and Egger (2010), Levy-Booth and Winder (2010) and Morales et al. (2010) we observed greater *nifH* abundance significantly associated with higher organic carbon (in our study OM), in addition to higher P and AS. Since N fixation requires large amounts of adenosine triphosphate (ATP) and reducing equivalents to fuel the process, heterotrophic diazotrophs are highly dependent on availability of carbon (Chan et al., 1997) and P, explaining the lower abundance of *nifH* in OM- and P-poor SL soil compared to C forest soil. The gene *nifH* gene was observed at greater abundance in microaggregates in the



**Fig. 6.** Principal component analysis performed on N cycling genes and other soil properties. Gene abundance included *nifH*, *nirS*, *niK*, *nosZ*, *amoA*-Arch, *amoA*-B and 16 s rRNA gene, and soil properties included SOC: soil organic carbon; N: Kjeldahl nitrogen; AP: available phosphorus; AS: aggregates stability; MBC: microbial biomass carbon; BSR: basal soil respiration. SL: soil under salvage logging treatment; C: control soil with tree retention.

degraded SL soil, but not in the control soil. The control soil has higher OM and AS (García-Orenes et al., 2017) possibly supporting greater *nifH* abundance throughout the intact top-soil, while in the SL soil the carbon loaded microaggregate fraction (Rabbi et al., 2016) possibly provides an advantageous niche for anaerobic N fixing bacteria. As suggested by Kennedy and Egger (2010), other types of N fixing microbes, such as photosynthetic cyanobacteria, may actually benefit from the loss of the tree canopy in SL soils since it allows greater light and increased soil temperatures (Ballard, 2000).

#### 4.2. Effect of post-fire treatment on denitrification genes abundance in soil

The nitrite reductase encoding genes, *nirK* and *nirS*, are often used as molecular markers of the denitrification process (Braker et al., 1998). In various natural environments, in particular aquatic environments, nirS has been found to dominate over nirK (Bothe et al., 2000; Liu et al., 2010; Throbäck et al., 2004; Oakley et al., 2007; Deslippe et al., 2014), however, nirK encoding the copper-containing nitrite reductase has been found to prevail in aerobic, oxygen-rich, environments (Desnues et al., 2007). In our study nirS and nirK were present in the soil at the same order of magnitude, however they showed different responses to SL. The abundance of these two genes is influenced by a range of factors, including soil moisture and temperature (Szukics et al., 2010; Rasche et al., 2011), total N concentration (Kandeler et al., 2009; Levy-Booth and Winder, 2010), concentration of available P, and soil OM (Petersen et al., 2012). García-Orenes et al. (2017) showed a decrease in total N, available P and soil OM in the SL managed soils. Although such reductions can explain a decrease in the abundance of the nirK and nosZ genes in post-fire SL soil, there was no correlation found between these parameters and *nirK* in our study. There were also differences in the distribution of these genes in the soil, with nosZ abundance being higher in the microaggregate fraction in both SL and control soils while *nirK* showed similar abundance in the intact soil and the microaggregate fraction. In agreement with Zhang et al. (2013), who reported mixed responses of denitrification genes, while nosZ and nirK showed a decrease in SL soils compared with C soil, the abundance of nirS was similar under all treatments.

The size of the denitrifying community may also indicate the greenhouse gas emission potential and aboveground net primary productivity of soils (Morales et al., 2010). The detection of *nosZ* in the soil suggests the presence of denitrifiers with the ability to reduce N<sub>2</sub>O to N<sub>2</sub> (Miller et al., 2008). Post-fire management, as our study showed, has the potential to greatly modify the size and structure of denitrifying bacterial communities in the soil, and could therefore have a great influence on gas emissions. This fact must be considered in current largescale models for climate change and other global phenomena (Morales et al., 2010).

#### 4.3. Effect of post-fire treatment on nitrification genes abundance in soil

The amoA gene has been used in molecular studies of AOB (ammonia-oxidizing bacteria) and AOA (ammonia-oxidizing archaea) communities (Norton et al., 2002). It has been reported that plant species, temperature, water content, C:N ratio and soil total N are strongly linked with the AOA and AOB community structure (Boyle-Yarwood et al., 2008; Rooney et al., 2010; Szukics et al., 2010; Rasche et al., 2011; Zeglin et al., 2011; Szukics et al., 2012). In our study post-fire SL significantly reduced the abundance of both archaeal and bacterial amoA (ammonia oxidisers) and both AOA and AOB amoA genes were found in higher concentrations in the microaggregate fraction than in the intact soil. Archaeal amoA genes have been found in diverse environments, for example oceans (Francis et al., 2007), and soils (Leininger et al., 2006; Di et al., 2010). AOB-amoA genes have been found to dominate over AOA-amoA in some soils (Di et al., 2010), while AOA-amoA is more abundant in other soils (Leininger et al., 2006). Interestingly, despite the requirements of less oxygen by AOA ammonia oxidisers (Levy-Booth et al., 2014 and references within), in our study they were found to be present in similar numbers, both concentrated in the soil microaggregates, and they were similarly affected by SL. The post-fire management, which has affected soil properties, especially plant species, water content by structure modification (field capacity), and C (carbon) and N content in the soil (García-Orenes et al., 2017), also influenced the abundance of archaeal and bacterial *amoA*. The AOB community responds to changes in pH and ammonia availability, both commonly increasing after forest fires, with pH increase being associated with enhanced bacterial *amoA* gene clusters (Levy-Booth et al., 2014). Both AOA and AOB are relatively slow growing (Prosser and Nicol, 2008), which could explain the lower overall abundance compared to other N-cyclers found in this and other work.

#### 5. Conclusions

Functional gene assessment can determine the relationship between microbial communities and ecosystem functions and can be a useful tool to select the optimal post-fire forest management. We have demonstrated in this work that post-fire management strategy, SL or C, significantly impacts on the community structure of N cycling microbes in forest soil. Soil microbes were present at higher abundance in C soil, where trees were retained post-fire, and in soil microaggregates. Soil properties that were affected by SL, in particular a reduction in OM, available P, N and AS, were accompanied by changes in microbial soil communities, including functional bacteria involved in N cycling.

Microbial gene pools involved in the N cycle, including N fixation, bacterial and archaeal nitrification and denitrification, recovered faster in control soil without post-fire tree removal than after SL. This fact is especially important in burned soil because improved N cycling could increase N pools available for re-vegetation following fire. This is supported by the control soil having higher plant richness and evenness than the soil following post-fire SL.

The importance of the microaggregate fraction for soil function is highlighted in this work with the microaggregates proving to be an important hot-spot for bacterial abundance, especially in the disturbed SL soil. The control soil with its organic matter and aggregate stability supported bacteria and N cyclers in the bulk soil, not only in the microaggregates.

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## HYDROLOGICAL RESPONSE 3 YEARS AFTER SALVAGE LOGGING TREATMENTS IN A RECENTLY BURNT FOREST SOIL

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Forest fires are common and must be considered as an ecological factor in the Mediterranean, however, especially in last five decades, changes in land use have provoked a modification in their natural regime. Moreover, post-fire management can have an additional impact on the ecosystem; in some cases, even more severe than the fire. Salvage logging is a common management technique in fire-affected areas, but carrying it out, using heavy machinery, with the consequent vulnerability of soils to erosion and degradation can damage soil and vegetation that is in a recovery state. We monitored some soil properties during more than 3 years in an area affected by a big forest fire (>500 has), which occurred in July 2012, where this post-fire treatment was applied. The study area is located in "Sierra de Mariola Natural Park" in Alcoi, Alicante (E Spain). The forest was composed mainly of *Pinus halepensis* trees with an understory of typical Mediterranean shrubs species such as Quercus coccifera, Rosmarinus officinalis, Thymus vulgaris, Brachypodium retusum, etc. Soil is classified as a Typic Xerorthent (Soil Survey Staff, 2014) developed over marls. In February 2013, salvage logging (SL) treatment consisting of a complete extraction of the burned wood using heavy machinery was applied in a part of the affected forest. Plots for monitoring were installed in this area and in a similar nearby area where there was no treatment, and which was then used as control (C) for comparison. Soil samplings were done immediately after treatment and every 6 months and some soil properties were analysed in laboratory. We found that all soil properties were negatively affected by SL treatment compared with C plots: a progressive decrease with time of organic matter content, microbial biomass, soil respiration, aggregate stability and an increase in bulk density (Mataix-Solera et al., 2015). This effect was also reflected in the abundance and diversity of plant species. In this work we observed the soil hydrological behaviour 3 years after treatments using the portable rainfall simulator designed by Kamphorst (1987). Rainfall simulations (n=15) were performed to study soil hydrological response comparing control plots (C), where no treatment was applied, over bare soil in salvage logging treatment area (BSL) which represents more than 50% of surface, and after applying a mulch of pine needles (MPN) in BSL area. Runoff and soil erosion rates were more than double in BSL with regard to C plots. MPN reduced the runoff to 50% with regard to BSL plots but far from the data registered in C plots. Results showed that in our study site, potential soil erosion is much higher in SL area than in C plots, where there was no human intervention.

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# Soil water retention curves and related properties two years after salvage logging treatments in a Mediterranean burnt forest

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## Abstract

In Mediterranean areas, water availability for plants is the main limiting factor for ecosystem restoration after fire. Post-fire management can have a negative impact on the soils that in some cases is even more severe than the fire itself. Salvage logging (SL) is a common management technique in fire-affected areas, but carrying it out, by using heavy machinery, leads to a consequent increase in the vulnerability to erosion and soil degradation. We monitored some soil properties in an area affected by a big forest fire (>500 has) in July 2012. The study area is located in the "Sierra de Mariola Natural Park", Alicante (E Spain). The forest was composed mainly of Pinus halepensis trees with an understory of typical Mediterranean shrub species. The soil was classified as a Typic Xerorthent developed from Miocene marls. In February 2013, the SL treatment, comprising a complete extraction of the burnt wood using heavy machinery, was applied in a part of the affected forest. Plots for monitoring were installed and in a similar nearby area there was no treatment (control, C) for comparison. Soil samplings were done immediately after treatment and every 6 months. We found that all soil properties were negatively affected by SL treatment compared with C plots: a progressive decrease with time of soil organic matter content, microbial biomass, soil respiration, aggregate stability, water holding capacity and an increase in bulk density were observed. In May 2014, undisturbed soil cores (100 cm<sup>3</sup>) from both treatments (C and SL) were taken in order to study water retention curves. Results showed differences between treatments, most likely due to differences in the pore size distribution of soils and the strong influence of parent material. The underlying marl rocks contain only very fine pores and thus they are "impermeable" once saturated with water. The water does not infiltrate into these rocks but flows down the surface and increases the erosion. The soil samples from SL treatments have higher content of coarse pores and thus they retain more water under wet conditions compared C samples where the humus horizon is preserved. But they have a lowered ability to retain water at high water tension in dry conditions. The soil samples from C plots showed the best properties. The soils have relatively stable structure and higher content of finer pores and thus the soil retains more water in dry conditions compared to the eroded soil at SL plots.

## Key words

Forest fire, salvage logging, water retention curves, aggregate stability, soil quality.

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Research article

## The role of mosses in soil stability, fertility and microbiology six years after a post-fire salvage logging management



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Keywords: Biocrust Mosses Wildfire Salvage logging Post-fire management Fertility

#### ABSTRACT

After a wildfire, moss crust develops in early post-fire stages revealing important roles related to soil erosion prevention and increase of soil fertility. However, the post-fire management selected could determine the capacity of soil to recover and the active role of mosses in the ecosystem recovery. Salvage logging (SL) was performed in the wildfire that occurred in July 2012 in "Sierra de Mariola Natural Park" (E Spain), with detrimental consequences to soils in the short-term. The aim of the study is to assess if the presence of a biocrust dominated by mosses six years after the wildfire improved the soil quality and functions, and if the salvage logging management influenced the process. Our results showed that the SL management affected in a mediumterm to the percentage of soil covered by mosses, reaching 78.4% in control soils compared to 56% in SL soils. Regarding the influence of mosses in soils, our results did not show greater differences in the physical parameters measured, hydraulic conductivity, water repellency and soil penetration resistance, possibly related to the lower developmental stages of the biocrust. However, it was observed that the presence of mosses played a significant role in both the soil fertility and the microbial activity. The improvement in soil fertility was registered mainly in the organic carbon, nitrogen, and phosphorous contents, and for the microbial parameters, for which higher values for the microbial biomass carbon and basal soil respiration were reached in soils under mosses. In conclusion, we can suggest that mosses had an important role in the functional recovery of degraded ecosystems after wildfires, and therefore we encourage considering the presence of mosses in the post-fire managements.

#### 1. Introduction

Biological soil crusts (BSCs) are a community of organisms composed mainly by cyanobacteria, lichens, and mosses, which inhabit the soil surface (Belnap and Lange, 2013). Although they are particularly important in drylands, BSCs are present in most environments. BSCs have key roles for the health and functionality of the ecosystems, improving soil structure and stability, influencing the local hydrologic cycles (Belnap, 2006), enhancing fertility and nutrient fixation (Belnap et al., 2001), increasing the biodiversity of soil microbial community (Xiao and Veste, 2017) and enabling plant establishment and growth (Muñoz-Rojas et al., 2018).

Biocrust are sensitive to disturbances, and wildfire induces severe impacts on them; biomass, cover and diversity use to decline dramatically (Johansen, 2001). Low-intensity fires were observed to have much less drastic effects (Bowker et al., 2004), although repeated burning by changes in fire regimes may destroy structures of soil organisms and threaten biocrust recovery (Greene et al., 1990; Hilty et al., 2004; Root et al., 2017). The recovery rates after a wildfire, in particular, will depend on fire severity and frequency, climatic conditions, topographical conditions, the availability of inoculant material and the adaptation of the biocrust community to fires (Weber et al., 2016). Mosses have been described as faster colonizers after wildfires creating a dense layer that precedes the establishment of vascular vegetation, also in the temperate Mediterranean forest (De Las Heras et al., 1994; Castoldi et al., 2013; Silva et al., 2019). This could suggest a relative role of mosses in early post-fire stages.

After a wildfire, the soil surface is exposed due to the absence or low vegetation cover (Bowker et al., 2008), very vulnerable to rainfall events, runoff and erosion processes (Chamizo et al., 2015). The presence of mosses in the first stages after wildfire provides high protection against the raindrop impacts and contributes to the roughening of the soil surface, which increases the water retention time hence leading to an improvement of the infiltration rates (Rodríguez-Caballero et al., 2003).

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2015). Moreover, soils covered by mosses register fertility increases (Guo et al., 2008). However, additional disturbances associated with the restoration methods could negatively affect the development of the biocrust and, ultimately, hinder ecosystem recovery (Hilty et al., 2004). Reductions in cover, richness, and diversity have been reported in mosses under different forest management in few studies: clear cutting (Paquette et al., 2016); harvesting (Caners et al., 2013), or salvage logging (Bradbury, 2006; Pharo et al., 2013).

Post-fire management is an important factor that will determine the capacity of soil to recover from degradation, together with fire history, ash properties, topography, post-fire weather, and vegetation recuperation (Pereira et al., 2018). Mediterranean ecosystems are resilient to fire disturbance. Hence, only when high severity fires could cause strong soil degradation, post-fire interventions should be planned to decrease the negative impacts, based on sustainable practices aimed at the maintenance and improvement of soil ecosystems services and to increase the resilience of forest to fire. Salvage logging (SL) is common management after a wildfire worldwide. However, multiple studies in the recent decades have reported negative impacts on plant regeneration and diversity (Beschta et al., 2004), soil erosion (Slesak et al., 2015), detrimental effects on microbial biomass and activity (Marañón-Jiménez et al., 2011), and impacts on nutrient cycling (Serrano-Ortiz et al., 2011; Pereg et al., 2018). SL can be very aggressive if soils are vulnerable to erosion and depending on the way to perform it. Whereas Francos et al. (2018) reported non-detrimental effects on the soil by manual log, the use of heavy machinery has reported adverse effects in several studies (Wagenbrenner et al., 2016; García-Orenes et al., 2017).

Little is known about the effects of the presence of a biocrust and their active role in the recovery of a soil affected by wildfire and post-fire management disturbances. Considering their highly relevant ecological functions, we hypothesize that the presence of a biocrust may improve the functional recovery of degraded soils, and thus, the ecosystem. For this purpose, we studied the role of a biocrust dominated by mosses in a soil affected by an aggressive post-fire salvage logging management six years after the disturbances (fire and SL). We assessed their influence in the soil attending to their role in soil stability studying relevant physical properties, their impact on soil fertility and the effects on the microbial activity. The aim of the research is to provide greater information to support the understanding of the function of mosses in the ecosystem recovery in order to improve post-fire management knowledge.

#### 2. Materials and methods

#### 2.1. Site description

The study area is located in "Sierra de Mariola Natural Park" in Alcoy, Alicante, in E Spain (38°43′59″ N, 0°29′16″ W). Climate in the area is Mediterranean, 3–4 months of summer droughts, dry-hot summer, and wet-warm spring, autumn and winter. The precipitation concentrates in October–November with an annual average of 490 mm, and mean temperature is 15.2 °C. *Pinus halepensis* Mill. trees around 40 years old are the main components of the forest, together with an understory of typical Mediterranean shrubs species such as *Quercus coccifera* L., *Rosmarinus officinalis* L., *Thymus vulgaris* L., and, *Brachypodium retusum* (Pers.) Beauv. The landscape is characterized by terraced hillslopes. The soil is classified as a Typic Xerorthent (Soil Survey Staff, 2014) developed over marls with low depth, very vulnerable to erosion and degradation processes, characterized by clay loam texture with 40, 25 and, 35% of sand, silt and clay respectively, and 40% of carbonates.

A wildfire occurred in July 2012 affecting a total of 546 ha. Fire severity was moderate according to Keeley (2009). All understory plants were charred or consumed, fine dead twigs on soil surface were consumed and log charred, also the pre-fire soil organic layer was largely consumed. Six months after the wildfire, in February 2013, SL management consisting of a complete extraction of the burned wood

using heavy machinery was applied in a part of the affected forest. Due to the vulnerability of soils, the management resulted in rills formation. At present, six years after, the affected soils are mostly covered by vegetation, but bare soils remain in the burned area as a symptom of degradation.

#### 2.2. Experimental design and sampling

Two adjacent study areas of  $2500 \text{ m}^2$  each were established, one of them where SL management, consisting of complete extraction of the burned wood using heavy machinery, was carried out, and the other in a similar nearby area where no intervention took place (all trees left standing), referred as control (C).

Previous to the soil sampling, a vegetation cover study was conducted in both areas using a  $50 \times 50 \text{ cm}^2$  quadrat divided into 100 cells. The quadrat was distributed randomly in the area managed by SL (n = 25) and control area (n = 25) to sampling total and moss covers.

For the study, we compared the soils covered by a dense layer of mosses to bare soils, or barely covered by vegetation, considered a symptom of remaining soil degradation (Fig. 1). For field measurements and soil sampling, 100 points were randomly distributed, 50 points for each management, SL and C, 25 replicates over mosses and 25 over bare soils for both management. Soil samples were taken from the upper soil layer (0–2.5 cm) since the influence of the mosses in soils is concentrated in the first centimeters of topsoil.

#### 2.3. Field measurements

A Mini-Disk Infiltrometer (MDI) (Decagon Devices, 2018) was used to measure hydraulic conductivity (K) due to its small size and easy handling (Robichaud et al., 2008), and according to its technical manual and equations proposed by Zhang (1997). The volume of water infiltrated was recorded every 30s and at least 10 min. Water repellency (WR) in soils and mosses was assessed in the field by the Water Drop Penetration Time (WDPT) test (Wessel, 1988), taking the average time for 5 drops as the WDPT value of a sample. Soil penetration resistance (PR), representing the pressure applied to the soil surface before it breaks, was measured in each point in 2 cm intervals by 5 pseudoreplicates, using a field penetrometer (Geotester Pocket Penetrometer, Italy) (Zaady and Bouskila, 2002).

#### 2.4. Laboratory analysis

Samples were divided into two subsamples, one subsample was stored at 4  $^{\circ}$ C and used for microbial analysis, and the other was airdried at room temperature (20  $^{\circ}$ C), during 1–2 weeks until constant



Fig. 1. Detail of the spatial distribution of mosses and bare soils in the field.

weight. A part was sieved between 4 and 0.25 mm to measure the aggregate stability (AS), and at < 2 mm for the chemical analysis.

Soil organic carbon (OC) was determined by the potassium dichromate oxidation method (Nelson and Sommers, 1982); total nitrogen was determined by the Kjeldahl method (Bremner and Mulvaney, 1982); and available phosphorus was determined by the Burriel-Hernando method (Díez, 1982). The aggregate stability (AS) was measured by the method of Roldán et al. (1994), based on the method of Benito and Díaz-Fierros (1989). This method examines the proportion of aggregates that remain stable after a soil sample is subjected to an artificial rainfall of know energy (279 J/min/m). Available micronutrients Fe, Zn, Cu, and Mn were extracted by DTPA in a soil:solution ratio 1:2 and 2 h shaking at room temperature, determined by atomic absorption spectrophotometry (AAS). The basal respiration (BR) of soil was measured in an automated impedance-meter (BacTrac 4200 Microbiological Analyser, Sylab, Austria), based on CO<sub>2</sub> emission by soil microorganisms at 30 °C for 24 h detected by indirect impedance measurement. Microbial biomass carbon (Cmic) was measured, also in the impedance-meter, as substrate-induced respiration with glucose (3 mg per gram of soil) as carbon substrate according to the Anderson and Domsch (1978).

#### 2.5. Statistical analysis

For the study of vegetation cover, non-parametric Mann-Whitney *U* test was performed due to the lack of normality and homoscedasticity requirements. In the case of soil parameters, normal distribution was checked by Shapiro-Wilks test and variables were transformed when necessary in order to assume statistical parametric assumptions. In order to study the effect of factors in soil parameters, a two-way ANOVA test

was performed considering two factors, management that include the levels SL and C, and the presence of not of a biocrust, with two levels, mosses (M) or bare soils (BS). Multiple comparisons were performed with Tukey's test (p < 0.05). A Principal Component Analysis (PCA) was performed to determine the effects of the management in the parameters analyzed. The physical parameters K, WR, PR, and AS were removed to simplify the overall analysis due to the low contribution with any principal components. The variables finally included in PCA were the chemical N, OC, P, Mn, Fe, Zn, Cu, and microbial Cmic and BR.

All these analyses were performed with a confidence level of 95% by Rstudio (2015) (Rstudio Team, Boston, USA), with "agricolae" package (De Mendiburu, 2019), and the PCA with "FactoMineR" (Le et al., 2008) and "factoextra" (Kassambara and Mundt, 2017) packages.

#### 3. Results

#### 3.1. Vegetation and moss cover dynamic

The study of vegetation in the area showed a high percentage of total cover, with significant differences between managements, 93.8% in SL and 99.8% in C soils (p < 0.05). Differences between moss cover were more noticeable (p < 0.05), 56% ( $\pm$  30.7) for SL in contrast to 78.4% ( $\pm$  29.7) of cover in the no intervention area. The elevate variance was due to the high spatial variability of mosses in the field for both areas (For more details see Table A1 SI).

#### 3.2. Physical properties

The parameters measured showed low differences between the



**Fig. 2.** Box-plot of (a) Hydraulic conductivity, K (mm/h); (b) water repellency, WR (s); (c) penetration resistance, PR (kg/cm<sup>2</sup>); and (d) Aggregate Stability, AS (%), measured in soils managed by salvage logging (SL) and control (C), on bare soils and on mosses. Points represent outliers, lowercase letters represent significant differences among mean groups (Tukey test P < 0.05).

managements and the presence or absence of mosses covering the soil (Fig. 2). The hydraulic conductivity (K) results did not show significant differences, however, there is a slightly increasing trend of this parameter appreciated over mosses. In addition, mosses developed WR (mean values 10 s), while bare soils hardly ever showed repellency. Nevertheless, the maximum value for WR was 60 s, reveling that drop penetration was relatively rapid on both, over mosses and bare soils.

The resistance to penetration (PR) did not show differences for the managements and soil cover (moss or bare soil), with very low values  $(1.2-1.5 \text{ kg/cm}^2)$ . The same happened for the percentage of aggregate stability (AS), but in this case with high levels of aggregation (around 75%).

#### 3.3. Soil fertility

The organic carbon, nitrogen and phosphorous contents concentrated in soils under mosses at depth 0–2.5 cm. The three parameters were significantly influenced by the presence of mosses covering the soil surface as we can see in Table 1. In addition, for OC and N, soils under mosses and without SL showed significantly higher concentration in relation to the managed: 3.16% in control soils in contrast to 2.36% in SL soils for the OC, and for N 2.26% in control soils and 1.99% for SL. In contrast, although higher values were recorded for P under mosses, values in SL were significantly higher than in C. An interaction between the two factors, management and presence of biocrust, was detected for the element.

Micronutrients showed different behavior among them (Table 1). Fe showed the same pattern as P, influenced by the presence of the biocrust, with higher values under mosses, significant in SL soils. Zn was also accumulated under mosses but only in SL soils, showing significant influence by the management applied. In contrast to Fe and Zn, Cu values were two-fold significantly lower in soils under mosses. On the other hand, Mn contents were highly influenced by the management applied, showing significantly higher values regardless of the presence of mosses or not, but an interaction between management and mosses was detected.

#### 3.4. Microbial parameters

Both microbial parameters measured showed the same pattern as can be observed in Fig. 3. Values for Cmic were significantly higher in soils under mosses, without differences between managements, 612 mg/kg in SL soils under mosses, two-fold higher than in bare soils (292 mg/kg), and 703 mg/kg in soils under mosses in C, more than 2.5 fold higher than in bare soils (267 mg/kg).

The microbial activity, reflected in the soil basal respiration (BR), showed significantly higher values in soils under mosses. No significant differences between managements were showed, 7.14  $\mu$ g/h/g dry soil in control and 7.99  $\mu$ g/h/g dry soil in SL, but with shorter differences with the bare soils in both cases.

#### 3.5. Multivariable analysis

The PCA showed chemical and microbiological properties explaining the relationships between the managements studied (Fig. 4). The model explained 70.2% of the total variation. In the first component, positively grouped with high loadings were N, OC, Cmic, highly influenced by the C management, and BR. Negatively correlated to them was Cu. In this first component, high loadings were also reached for Fe and P, mainly explained for the SL management. This also occurred for Mn and Zn variables grouped together in the second component, with the highest loading for the Mn element.

#### 4. Discussion

Post-fire management selection will be crucial in how the ecosystem

#### Table 1

Mean and standard deviation values measured in soils managed by salvage logging (SL) and control (C) (factor management), on bare soils and on mosses (factor biocrust), two-way ANOVA results and significant differences at  $p < 0.05^*$ ,  $p < 0.01^{**}$  and  $p < 0.001^{***}$ . n.s. not significant at a p < 0.05. Lowercase letters represent significant differences among mean groups (Tukey test P < 0.05). (OC: organic carbon; N: total nitrogen; P: available phosphorous; Fe: iron; Zn: zinc; Cu: copper; Mn: manganese).

	Management	Biocrust	Mean	sd	Factors	p- Value
OC	С	Bare Soil	2.44 <sup>a</sup>	0.87	Management	***
(%)		Mosses	3.16 <sup>b</sup>	0.83	Biocrust	***
	SL	Bare Soil	1.90 <sup>a</sup>	0.61	Manag: Biocrust	n.s.
		Mosses	2.36 <sup>a</sup>	0.79		
N	С	Bare Soil	1.79 <sup>ab</sup>	0.56	Management	*
(%)		Mosses	2.26 <sup>c</sup>	0.55	Biocrust	***
	SL	Bare	1.56 <sup>a</sup>	0.41	Manag:	n.s.
		Soil			Biocrust	
		Mosses	1.99 <sup>bc</sup>	0.55		
Р	С	Bare Soil	6.15 <sup>a</sup>	4.37	Management	n.s.
(mg/ kg)		Mosses	7.99 <sup>ab</sup>	4.24	Biocrust	***
	SL	Bare	5.67 <sup>a</sup>	1.29	Manag:	*
		Soil Mosses	11.36 <sup>b</sup>	7.03	Biocrust	
Fe	С	Bare Soil	1.09 <sup>a</sup>	0.33	Management	n.s.
(mg/ kg)		Mosses	1.46 <sup>ab</sup>	0.62	Biocrust	***
-	SL	Bare Soil	1.25 <sup>a</sup>	0.43	Manag: Biocrust	n.s.
		Mosses	$1.75^{\mathrm{b}}$	0.92		
Zn	С	Bare Soil	0.33 <sup>a</sup>	0.22	Management	**
(mg/ kg)		Mosses	0.32 <sup>a</sup>	0.16	Biocrust	n.s.
	SL	Bare Soil	0.38 <sup>ab</sup>	0.11	Manag: Biocrust	n.s.
		Mosses	$0.53^{b}$	0.30		
Cu	С	Bare Soil	0.19 <sup>b</sup>	0.09	Management	**
(mg/ kg)		Mosses	0.11 <sup>a</sup>	0.06	Biocrust	***
	SL	Bare Soil	0.26 <sup>c</sup>	0.08	Manag: Biocrust	n.s.
		Mosses	0.12 <sup>c</sup>	0.07		
Mn	С	Bare Soil	0.42 <sup>ab</sup>	0.55	Management	***
(mg/ kg)		Mosses	0.16 <sup>a</sup>	0.13	Biocrust	n.s.
0-	SL	Bare Soil	0.90 <sup>b</sup>	0.44	Manag: Biocrust	*
		Mosses	0.97 <sup>b</sup>	0.39		

functionality will recover after a fire disturbance. Salvage logging practice has reported in several works detrimental effects that hamper the ecosystem recovery (Lindenmayer and Noss, 2006; Leverkus et al., 2014). In a study carried out in the same area by García-Orenes et al. (2017), after two years of monitoring since the management, the affected area registered adverse effects on the vegetation recovery, together with general soil degradation. Six years after the wildfire, our findings suggest that both areas, SL and C, are mostly recovered showing high levels of soils cover by vegetation (see also in Table A2 SI). However, a high percentage of soils in SL were barely covered by dense vegetation, which is a symptom of degradation compared to C soils, a consequence of a very aggressive soil. In addition, the post-fire SL was found to have a medium-term impact on the moss recovery. The



Fig. 3. Box-plot of (a) Microbial biomass carbon, Cmic (mg/kg), and (b) soil basal respiration, BR ( $\mu$ g/h/g dry soil), measured in soils managed by salvage logging (SL) and control (C), on bare soils and on mosses. Points represent outliers, lowercase letters represent significant differences among mean groups (Tukey test P < 0.05).



Fig. 4. Scores and loadings for PCA performed for control soils (blue circles) and salvage logging soils (yellow triangles). (OC: organic carbon; N: total nitrogen; P: available phosphorous; Fe: iron; Zn: zinc; Cu: copper; Mn: manganese; Cmic: microbial biomass carbon; BR: soil basal respiration). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

percentage of cover could be decisive to reduce runoff since recent studies (Gao et al., 2019; Silva et al., 2019) suggest threshold values from which the presence of mosses could significantly reduce the sediment losses after the rain season. The negative effects on the recovery rate of bryophytes (liverworts, hornworts, and mosses) by SL management have been reported in other studies (Bradbury, 2006; Caners et al., 2013), highlighting the importance of maintaining the burned structures on topsoil since these acts as substrates that provide abundant resources to allow rapid dissemination.

Attending the physical parameters measured in soils and mosses, no greater differences were found six years after the disturbance. Immediately after the fire, the hydraulic properties of soils suffered important changes that can trigger erosion processes, due to the removal of protective vegetation, burning of organic matter that aggregates the soil and water repellency development (Shakesby, 2011). However, in medium-term hydraulic conductivity values did not show marked differences, which is in accordance with Robichaud et al. (2013) who using the MDI found that infiltration rates tend to recover in a short time after the wildfire. Nevertheless, the effects on the physical properties of SL are high depending on the type of soil and the equipment used (Fernández and Vega, 2016; Lucas-Borja et al., 2019).

Disturbances in BSCs caused by logging and fire have demonstrated to increase soil erosion (Wilson, 1999). The main reason is BSCs play critical roles in soil stabilization and erosion prevention, especially by influencing in different aspects the hydrological cycles: modifications in soil porosity, absorptivity, roughness, aggregate stability, texture, and water retention (Belnap, 2006). The pass of the time affects how BSCs influence hydrological processes (Chamizo et al., 2016), therefore the early presence of mosses after the fire presumably helped to control the erosion by covering the soil. But 6 years after the disturbance may not be enough time to develop higher biomass and a complex structure that could significantly affect the hydraulic conductivity (Faist et al., 2017), although a tendency is marked.

Our soils rarely showed water repellency, in contrast to mosses with slight WR times, also reported in other studies (Kidron et al., 2010; Moore et al., 2017). The hydrophobicity expressed by mosses, combined with pore clogging, could generate important runoff responses depending on the rainfall intensity (Xiao et al., 2011). However, the slight hydrophobic characters shown in the study could have a positive effect and help to trap water near the soil surface enhancing infiltration (Faist et al., 2017), reducing losses by evaporation.

In the case of surface penetrability, our soils did not present soil sealing and physical crusting, showing low PR values. For BSCs, higher developmental stages are related to higher penetration resistance (Zaady et al., 2014; Chamizo et al., 2015). Therefore, six years after a perturbation may not be enough time to develop a complex state of the biocrust that could reach higher levels of compaction comparing to bare soils. Similar happened with the AS parameter. In a previous work (García-Orenes et al., 2017), a decrease of nearly 15% in AS was registered two years after the fire in soils affected by SL. A decrease in the aggregate stability of soil due to the combustion of organic matter after a fire can significantly trigger runoff processes (Mataix-Solera et al., 2011; Shakesby, 2011). At present, AS values have recovered due to the high content of organic carbon (2–4%) in the forest soils, reaching similar values to unburned soils in this area (Chrenková et al., 2014; Jiménez-Pinilla et al., 2016).

Severe events of wind and water erosion after a wildfire usually involve important soil losses with high quantities of nutrients. In our soils, the fast colonization of mosses covering the soil helped to retain ashes into the soil in multiple samples (personal observations), which is an essential process to boost the short and long-term ecosystem recovery after a wildfire (Caon et al., 2014). BSCs have reported increasing soil nutrients, considered patches of fertility in drylands (Belnap and Lange, 2013). In our results, soils under mosses showed a significant increase of nutrients compared to bare soils. Together with the retention of ashes into the soil, their ability to transform organic matter is related to the increase of nutrients in soils. In this sense, higher OC and N content linked to a higher developmental of mosses showed a significant correlation in the PCA in soils. The increase of fertility in soils, due to the presence and development of BSCs, has been also corroborated by multiple studies, especially for total nitrogen (DeFalco et al., 2001; Barger et al., 2016), available phosphorous (Jafari et al., 2004; Guo et al., 2008) and organic carbon content (Kidron et al., 2010; Yang et al., 2019).

Results for micronutrients were variable. While Fe and Zn concentration could be explained by the presence of mosses, Mn and Cu showed different behavior. Apart from trapping ashes into the soil in early postfire stages, other mechanisms of BSCs to increase micronutrient fertility is via dust trap, fine particles, clay, silt, and possibly ash particles (Belnap et al., 2001). The roughness of mosses in the soil surface helps to incorporate the dust, whose nutrients positively charged remain bonding to negatively charged clay particles (Belnap and Harper, 1995). Indeed, recent publications highlighted the considerable ability of mosses to accumulated trace elements in different ambient, including Fe, Mn, Cu and Zn (Bidwell et al., 2019; Wang et al., 2019). Higher values for Zn, Fe, and Mn (also for P) in SL soils, correlations showed in the PCA, could be due to the higher area of bare soils exposed, thereby more dust particles in the air and finally trapped over the mosses for some of the elements. In contrast, Cu values were lower under mosses, reported in Beraldi-Campesi et al. (2009) as leaching effects for many metals and metalloids (also Cu) promoted by the activity of the microorganisms, hypothesis that will need direct testing in the future. Nevertheless, concentrations for all micronutrients were low in our soils (Kabata-Pendias, 2011).

Mosses covering the soil showed a clear effect on the microbial parameters measured. Biologic parameters are expected to increase under

BSCs, influenced by the improvement of soil parameters like the organic matter content, the activity, and abundance of microorganism activity will be boost (Belnap and Harper, 1995). However, the improvement is related to the age since the disturbance, progressively increasing with time (Liu et al., 2017). In our results, a significant improvement of the microbial parameters under mosses was found, being this related to the increase in OC and N in soils observed in the PCA analysis, a correlation that is stronger for Cmic in C soils. In García-Orenes et al. (2017), short-term results showed low microbial biomass and activity after the fire, since microbiological parameters respond very quickly to perturbations in soil (Mataix-Solera et al., 2009), nonetheless recovery of soils affected by SL was much slower compared to soils with no intervention. Presumably, Cmic will significantly increase with time together with the improvement of the chemical parameters related to the fertility of soils. Multiple studies recommend the maintenance of the burn wood (total or partially) on the topsoil to create microclimatic conditions enhancing soil moisture; thereby promote microorganisms development (Marañón-Jiménez et al., 2011). Future research should examine soil microbial functions under mosses to better understand their role in microbial improvement.

Given their relevant role in soils after a wildfire, mosses should be considered in the post-fire management plans. Ensure their development and dispersal is a restoration strategy to take into account, assisted by the preservation of woody substrates as provisions and controlling the managements like salvage logging. However, in agreement with Silva et al. (2019), the feasibility as a practical post-fire measure still needs to be proved, partly since mosses respond to different ambient conditions. Nevertheless, the interest in using BSCs as biotools in restoration is becoming increasingly relevant and a reality (Maestre et al., 2017; Bethany et al., 2019).

#### 5. Conclusions

Salvage logging, a practice that has been demonstrated to be aggressive with soils in the short-term, has affected in a medium-term to the percentage of soil covered by mosses. In this study, we observed that the soils covered by a biocrust dominated by mosses showed better conditions since protected from erosion the soils showed better soil quality, hence creating better conditions for soil functions. The presence of mosses promotes soil recovery after a severe disturbance like a wildfire continued by aggressive post-fire management such as salvage logging. The improvement has been registered mainly in the fertility characteristics and microbial parameters. The physical parameters have not registered greater differences, and we hypothesize that mosses have key roles in early post-fire stages but more developmental stages of the biocrust and time will be necessary to register more differences in those parameters.

Considering their relevant ecological functions, such as increasing the stability of surface soils, improving soil fertility, and microbial activity, mosses could be key in the functional recovery of degraded ecosystems after wildfires. For this reason, mosses should be considered in the post-fire management. Therefore, we recommend to forest managers to take into account the natural recovery of mosses in order to do not affect them if any post-fire management is planned, thereby develop a sustainable forest that increases biodiversity and resilience. Moreover, the maintenance of the burnt wood could have long-term consequences for the conservation of biocrust and mosses.

#### CRediT authorship contribution statement

Minerva García-Carmona: Conceptualization, Methodology, Investigation, Writing - original draft, Writing - review & editing. Victoria Arcenegui: Methodology, Investigation, Writing - review & editing. Fuensanta García-Orenes: Conceptualization, Methodology, Writing - review & editing, Project administration, Funding acquisition. Jorge Mataix-Solera: Conceptualization, Methodology, Writing - review & editing, Project administration, Funding acquisition.

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#### Appendix A. Supplementary data

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# Los musgos promueven la recuperación de la comunidad microbiana en suelos afectados por tala y saca de madera seis años después del incendio

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## Resumen

Los manejos post-incendio pueden tener un impacto adicional en el ecosistema, siendo en algunos casos incluso más severos que el propio incendio. En la Sierra de Mariola (Alicante) se llevó a cabo un tratamiento de tala y saca de madera, SL (del inglés *Salvage logging*), al poco tiempo de producirse un incendio, resultando en un detrimento de múltiples propiedades del suelo. El objetivo de este trabajo fue evaluar los efectos a medio plazo del manejo SL, y la influencia de los musgos que cubren el suelo, en la recuperación de las propiedades biológicas. La estructura microbiana está estrechamente relacionada con la calidad del suelo y el mantenimiento de los servicios ecosistémicos, y en este sentido, los cambios en la estructura microbiana del suelo pueden evaluarse a través del análisis de PLFAs. Nuestros resultados mostraron consecuencias negativas significativas en cuanto al manejo SL, pero la presencia de musgos presumiblemente promovió la recuperación de la abundancia y diversidad de la comunidad microbiana. Por tanto, dado su papel significativo en el suelo, recomendamos tener en cuenta a los musgos en cualquier manejo post-incendio.

Palabras clave: incendio forestal, tala y saca de madera, musgos, PLFAs, estructura microbiana.

## 1. Introducción

Los incendios forestales a menudo cambian las propiedades de los suelos, principalmente debido a la destrucción de la cobertura de la vegetación y la pérdida de la materia orgánica. La tala y saca de madera (SL), es un tratamiento muy común en las áreas afectadas por incendios, la cual consiste en la extracción de la madera quemada, en muchos casos usando maquinaria pesada. Llevar a cabo el SL en este estado puede incrementar la perturbación y la erosión, comprometiendo la recuperación de la vegetación, y afectado negativamente a diversas propiedades del suelo, especialmente las propiedades biológicas (García-Orenes *et al.*, 2017).

Los musgos han sido descritos como colonizadores tempranos tras incendios creando una capa densa que precede al establecimiento de la vegetación vascular, lo cual puede sugerir un papel clave en estadios tempranos post-incendio. Los musgos forman parte de las costras biológicas del suelo (CBS), una comunidad de organismos que habitan la superficie de los mismos. Existe una extensa bibliografía sobre los efectos positivos de estos organismos para la salud y funcionabilidad de los ecosistemas: mejoran la estructura y estabilidad del suelo, favorecen la fertilidad y la fijación de nutrientes y facilitan el establecimiento de la vegetación vascular

(Belnap y Lange, 2013). Las CBS crean un microhábitat favorable para los microorganismos, incrementando así la biodiversidad de la comunidad microbiológica del suelo (Xiao y Veste, 2017). Múltiples procesos clave en el ecosistema, como la descomposición de la materia orgánica, el secuestro de carbono e incluso el ciclo del agua, se ven beneficiados de la abundancia y diversidad de los microorganismos que forman parte de las CBS.

En este estudio, planteamos la hipótesis de que las CBS compuestas por musgos tuvieron un papel clave en la recuperación de las propiedades microbiológicas del suelo, y por tanto de la funcionabilidad del ecosistema, después de importantes perturbaciones como son un gran incendio forestal y un manejo post-incendio muy agresivo con el suelo. Con el objetivo de estudiar los efectos a medio plazo (6 años) del manejo SL sobre los suelos, y el impacto de la presencia de musgos, se evaluaron en el suelo la estructura microbiana, el carbono de la biomasa microbiana (Cmic) y la respiración edáfica basal (RB), junto con el carbono orgánico (CO), como factores clave a la hora de controlar la calidad de un suelo.

## 2. Materiales y Métodos

## Área de estudio y diseño experimental

El estudio se localiza en el Parque Natural de "Sierra de Mariola" en Alcoy, Alicante (España). El clima es mediterráneo, con una precipitación media anual de 490 mm y temperaturas de 14.8°C. El suelo está desarrollado sobre margas, muy vulnerable a los procesos erosivos, caracterizado por un 4.6% de materia orgánica en los primeros 5 cm de suelo, textura franco arcillosa (40, 25 y 35% de arena, limo y arcilla respectivamente) y un 40% de carbonatos.

Se establecieron dos áreas de 50x50 m<sup>2</sup>, una tratada con SL y la otra cercana pero sin tratamiento, el control (C). En cada una de ellas se distribuyeron al azar 5 puntos sobre suelo desnudo y 5 sobre musgos (10 por cada tratamiento, 20 puntos en total).

## Análisis

Para medir los cambios en la comunidad microbiológica se llevó a cabo el análisis de ácidos grasos derivados de fosfolipídos (PLFAs) procedentes de las membranas de los microorganismos del suelo, según Bossio *et al.* (1998). Los ácidos grasos se extraen de 12 g de suelo usando un buffer de cloroformo:metanol:fosfato, y se separan de los ácidos grasos neutros y glicolípidos mediante una columna. Las muestras son analizadas en cromatografía de gases después de una metilación alcalina. Los ácidos grasos resultantes son proporcionales al contenido en bacterias, hongos, bacterias Gram-positivas y Gram-negativas y actinobacterias.

El carbono orgánico (CO) se determinó por el método de oxidación del dicromato potásico (Nelson y Sommers, 1982). La respiración basal (RB) se midió en un respirómetro de impedancia (BacTrac 4200 Microbiological Analyser, Sylab) basado en la emisión de CO<sub>2</sub> por los microorganismos tras 24h a 30°C. El carbono de la biomasa microbiana (Cmic) se midió por respiración inducida con glucosa (3 mg por g de suelo) conforme Anderson y Domsch (1978).

## 3. Resultados y Discusión

Se observó un efecto negativo del manejo SL sobre la mayoría de los grupos y total PLFAs (Figura 1). Por otro lado, el efecto de la presencia de musgos en los grupos microbiológicos fue claro. Los valores de abundancia significativamente más altos se encontraron en los suelos C cubiertos por musgos, excepto para el grupo Gram-, aunque hay una tendencia marcada.



**Figura 1.** Valores en nmol/ g suelo de PLFAs totales y biomarcadores (media ± desviación estándar) para tala y saca de madera (SL) y suelos control (C), en suelo desnudo (SD) o bajo musgos (M). Las letras indican diferencias significativas entre suelos (test de Tukey, p<0.05, tras ANOVA de una vía)

La correlación de Pearson (Tabla 1) mostró influencias significativamente positivas para el CO y Cmic para la mayoría de grupos y los PLFAs totales, excepto para las Gram-. Por otro lado, la RB no se relacionó significativamente con ningún grupo funcional.

			<i>,</i>	0		1
	Gram+	Gram-	Hongos	Bacterias	Actinobacterias	Total
СО	0.499*	0.039	0.428	0.471*	0.483*	0.491*
RB	0.324	0.078	0.247	0.329	0.254	0.309
Cmic	0 5/3*	0 173	0 487*	0 571*	0 507*	0 567*

**Tabla 1.** Coeficientes de correlación de Pearson (CO: carbono orgánico; RB: respiración basal; Cmic:carbono de la biomasa microbiana). Correlaciones significativas marcadas a p<0.05\*</td>

Los análisis de PLFAs revelan marcadas diferencias en relación al manejo y la presencia de musgos en los suelos 6 años después de las perturbaciones. La limitación en el desarrollo en los grupos microbianos debido a SL responde a que los que los microorganismos son muy sensibles a cambios en el manejo del suelo (Pascual-Rico et al., 2018). Por el contrario, los suelos sin intervención (C) demostraron un efecto positivo en la estructura microbiana, lo que posiblemente se deba al mayor contenido en CO y Cmic, altamente correlacionado con los diferentes grupos microbianos. En particular, las Gram+ dominan la población bacteriana, lo que según Bárcenas et al. (2011) es debido a que éstas son colonizadoras rápidas y de menor sensibilidad, y por tanto podrían verse estimuladas por la presencia de musgos y la mejora del suelo asociado en suelos sin SL. En contraste, las Gram- mostraron menor abundancia sin diferenciación entre tratamientos, lo que probablemente se relacione con el tipo de requerimiento de estos organismos, altamente dependientes de carbono orgánico lábil (Paul y Clark, 1996), profundamente afectado por el incendio. Se detectó una recuperación importante también para los hongos en C y bajo musgos, a pesar de que se recuperan menos rápido que las bacterias al ser más sensibles (Zornoza et al., 2009), pero la presencia de musgos parece que ha sido decisiva para su recuperación tras el incendio.

Por tanto, la presencia de musgos junto con la no intervención, parecen ser las mejores condiciones para la recuperación de la abundancia y diversidad de la comunidad microbiana del suelo. Los musgos son responsables de la estabilización del suelo y retención de agua, lo

cual promueve la mejora de múltiples propiedades del mismo. La regulación del ciclo de nutrientes, mineralización y estabilización del carbono posiblemente se vean beneficiados de la comunidad microbiana asociada a los mugos. Por esta razón las CBS podrían ser fundamentales para la restauración de un suelo afectado por el fuego y degradado.

## 4. Conclusiones

Seis años después del incendio y el manejo post-incendio, los resultados muestran un impacto negativo en la comunidad microbiana edáfica debido al manejo. SL fue muy agresivo con el suelo, pero el rápido desarrollo de los musgos podría tener un claro efecto en promover la recuperación de la microbiología del suelo, además de otras propiedades. De acuerdo a nuestros resultados, recomendamos tener en cuenta la recuperación natural de los musgos al planificar cualquier manejo post-incendio.

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