

Innovación en técnicas de plantación forestal contra la sequía y la vegetación competitiva en condiciones mediterráneas

Jaime Coello Gómez

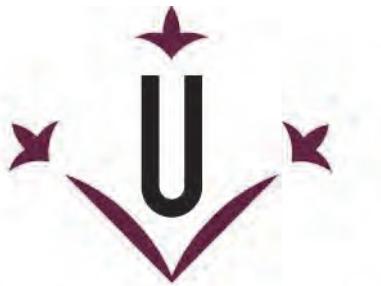
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Universitat de Lleida

TESI DOCTORAL

Innovación en técnicas de plantación forestal contra la sequía y la vegetación competitiva en condiciones mediterráneas

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Resum en català

La plantació d'arbres pot atendre a una gran varietat d'objectius productius, socials i ambientals. En aquest treball s'han assajat, en condicions climàtiques molt contrastades, un conjunt de tècniques innovadores contra la sequera i la vegetació competitora, dues de les principals amenaces per a un arbre jove en condicions mediterrànies. Les tècniques són:

- i) Condicionadors del sòl: barreges sinèrgiques de polímers hidroabsorbents amb ingredients que incrementen la fertilitat del sòl. Aquesta tècnica busca millorar la microestació en la qual s'instal·len els plançons.
- ii) Cobertes del sòl: superfícies opaques instal·lades al voltant del plançó que impedeixen la instal·lació i proliferació de vegetació competitora.

Els models específics avaluats busquen aconseguir resultats millorats en comparació amb tècniques de referència, des d'un punt de vista tècnic, ambiental i social.

S'ha provat amb èxit un nou condicionador del sòl en condicions semiàrides (amb pi blanc) i montanes (amb freixe). Les cobertes del sòl han afavorit l'establiment de plantacions de noguera en condicions productives (supramediterrani subhumid), tot i que el seu efecte en zones limitadores ha estat poc evident. Els models innovadors (biodegradables o realitzats amb materials reciclats) han mostrat ser una alternativa d'interès a les cobertes plàstiques de referència.

Aquestes noves tècniques representen per tant una opció a tenir en compte en futurs projectes de plantació forestal, en millorar els resultats en la crítica fase d'establiment.

Resumen en castellano

La plantación de árboles puede atender a una gran variedad de objetivos productivos, sociales y ambientales. En este trabajo se ha ensayado, en condiciones climáticas muy contrastadas, un conjunto de técnicas innovadoras contra la sequía y la vegetación competitiva, dos de las principales amenazas para un árbol joven en condiciones mediterráneas. Las técnicas son:

- i) Acondicionadores del suelo: mezclas sinérgicas de polímeros hidroabsorbentes con ingredientes que incrementan la fertilidad del suelo. Esta técnica busca mejorar la microestación en la que se instalan los brizales.
- ii) Cubiertas del suelo: superficies opacas instaladas alrededor del brizal que impiden la instalación y proliferación de vegetación competitiva.

Los modelos específicos evaluados buscan conseguir resultados mejorados en comparación con técnicas de referencia, desde un punto de vista técnico, ambiental y social.

Se ha probado con éxito un nuevo acondicionador del suelo en condiciones semiáridas (con pino carrasco) y montanas (con fresno). Las cubiertas del suelo favorecieron el establecimiento de plantaciones de nogal en condiciones productivas (supramediterráneo subhúmedo), aunque su efecto en zonas limitantes ha sido poco evidente. Los modelos innovadores (biodegradables o realizados con materiales reciclados) han mostrado ser una alternativa de interés a las cubiertas plásticas de referencia.

Estas nuevas técnicas representan por tanto una opción a tener en cuenta en futuros proyectos de plantación forestal, permitiendo mejorar los resultados en la crítica fase de establecimiento.

Summary in English

Tree planting can respond to a wide variety of productive, environmental and social aims. In this work we tested, in contrasting climatic conditions, various innovative planting techniques aiming at tackling the negative impact of drought and competing vegetation. These factors are two of the main threats for a young seedling in Mediterranean conditions. These techniques are:

- i) Soil conditioners: synergistic mixtures of water-absorbing polymers with ingredients increasing soil fertility. This technique aims at improving the micro-site conditions where the seedling is planted.
- ii) Mulches: opaque layers displayed on the ground around the seedling to prevent the establishment or proliferation of competing vegetation.

The specific models developed aim at improving the results of reference techniques from a technical, environmental and social point of view.

The soil conditioners led to very positive results in semiarid (with Aleppo pine) and Montane (with ash) conditions. The mulches improved seedling establishment of walnut in productive conditions (supramediterranean subhumid), although they had little effect in limiting sites. The innovative models (either biodegradable or made of recycled materials) seem to be a suitable alternative to reference plastic mulches.

These new techniques represent an alternative to consider in future tree planting projects, to improve the results in the critical phase of seedling establishment.

1. Introducción y objetivos

1.1. La plantación forestal: un reto global y mediterráneo

A nivel mundial se calcula que hay más de 2 millones de hectáreas degradadas por restaurar (Potapov *et al.*, 2011). Una de las principales herramientas de restauración de ecosistemas degradados es la plantación de árboles y arbustos (Berrahmouni *et al.*, 2015). Esta actividad se puede realizar con finalidad productiva (generación de productos, madereros o no), protectora (biodiversidad, suelos, aguas o infraestructuras), ornamental y social (bosques urbanos, parques y jardines), entre otras (McGuire, 2014). Sin embargo, el alto coste de la plantación de árboles es el principal factor que limita su implementación, lo que conduce a la búsqueda continua de técnicas de plantación forestal coste-eficientes (Oliveira *et al.*, 2011). Otras características apreciadas de una técnica de plantación forestal son su funcionamiento autónomo y prolongado en el tiempo, así como su bajo impacto ambiental considerando su ciclo de vida completo (Coello y Piqué, 2016).

En condiciones mediterráneas las principales amenazas para una plantación forestal recién instalada son la sequía y la vegetación competitora, además de los daños causados por la fauna. La disponibilidad de agua es, de hecho, el principal factor limitante de la producción primaria en el Mediterráneo (Villar-Salvador *et al.*, 2012), especialmente durante la estación seca, que coincide con la época de mayor temperatura. Además, las proyecciones climáticas auguran un incremento del estrés hídrico en el futuro, especialmente ligado al aumento esperado de las temperaturas (Vallejo *et al.*, 2012). La disponibilidad hídrica está condicionada por el régimen de precipitación y temperatura, pero también por la capacidad de retención de agua del suelo (definida en gran parte por su textura, pedregosidad y contenido de materia orgánica), la fisiografía (pendiente, orientación, concavidad o convexidad vertical y horizontal) y por otros aspectos como la insolación o el viento (Manrique-Alba *et al.*, 2017). La vegetación competitora hace referencia a aquellas especies vegetales que crecen de manera espontánea en el área plantada, ejerciendo una competencia por la luz, el agua y los nutrientes, por lo que pueden exacerbar el impacto negativo de la sequía (Rey Benayas *et al.*, 2003). Este trabajo se centra en estas dos amenazas interrelacionadas, sequía y vegetación competitora, a las que una plantación forestal es especialmente sensible durante los primeros años, en los que el sistema radical no está adecuadamente desarrollado para explorar los horizontes profundos del suelo (Vallejo *et al.*, 2012) y durante los cuales la copa no tiene aún capacidad para sombrear el suelo limitando el desarrollo de la vegetación competitora (Devine *et al.*, 2007).

1.2. Técnicas contra la sequía en plantaciones forestales

La principal técnica aplicada tradicionalmente contra la sequía en plantaciones forestales en el mediterráneo son los riegos de apoyo (Carminati *et al.*, 2010). Sin embargo, esta técnica presenta importantes limitaciones, entre las que destacan su alto coste, la dificultad de aplicación en terrenos de mala accesibilidad o transitabilidad y la dificultad para prever la necesidad de aplicación (Coello y Piqué, 2016). Las principales técnicas alternativas o complementarias al riego son i) la preparación del suelo en profundidad y/o modificando la microtopografía para concentrar la precipitación y la escorrentía; ii) técnicas viverísticas para generar brizales con un gran volumen o profundidad radical; y iii) la aplicación de acondicionadores del suelo (Cortina *et al.*, 2011; Löf *et al.*, 2012; Hüttermann *et al.*, 2009). Esta última técnica consiste en mejorar las propiedades físicas y/o químicas del suelo en el volumen del hoyo de plantación para facilitar así el desarrollo inicial del brinal (Coello y Piqué, 2016). Este producto se aplica manualmente en el momento mismo de plantar o sembrar. Uno de los componentes más exitosos de los acondicionadores del suelo son los polímeros hidroabsorbentes o hidrogeles, capaces de absorber 400 veces su peso en agua (Rowe *et al.*, 2005), quedando ésta a disposición de la planta durante más tiempo (Hüttermann *et al.*, 2009) al reducirse las pérdidas por infiltración o evaporación. La composición de estos polímeros incluye habitualmente poliacrilamida reticulada, un producto que despierta una crítica social creciente debido a las posibles trazas del monómero (acrilamida), que es tóxico. Aunque los fabricantes son capaces de producir poliacrilamida con una concentración de acrilamida libre dentro de los límites legales (Rowe *et al.*, 2005), existe una demanda creciente de hidrogeles sin poliacrilamida (DRI, 2008). Los acondicionadores del suelo pueden comercializarse en forma de hidrogel puro o bien como mezclas sinérgicas de hidrogeles con otros ingredientes que aportan beneficios adicionales, principalmente, fertilizantes (Rowe *et al.*, 2005; Soler-Rovira *et al.*, 2006).

El principal interés de los acondicionadores del suelo que incluyen hidrogeles es, por tanto, el incremento de la capacidad de retención de agua del suelo, habiéndose observado incrementos de supervivencia, crecimiento e indicadores fisiológicos en brizales de diversas especies de *Citrus sp.* (Arbona *et al.*, 2005), *Fagus sp.* (Beniwal *et al.*, 2011; Jamnicka *et al.*, 2013), *Pinus sp.* (Hüttermann *et al.*, 1999), *Quercus sp.* (Chirino *et al.*, 2011) y *Populus sp.* (Shi *et al.*, 2010). Además, en el caso de los acondicionadores que mezclan hidrogeles y fertilizantes se ha observado un incremento de los nutrientes del suelo (Machado *et al.*, 2016).

Los acondicionadores del suelo han mostrado de manera consistente su interés en condiciones de vivero. Sin embargo, en muchos estudios de campo este producto presenta un efecto poco apreciable (Clemente *et al.*, 2004; Chirino *et al.*, 2011) o incluso negativo (Barberá *et al.*, 2005). Una particularidad de esta técnica es que su efectividad depende en gran medida del tipo de suelo, de la formulación y de la dosis de aplicación. Los mejores resultados se obtienen en suelos de textura ligera, es decir, arenosos o franco-arenosos (Koupai *et al.*, 2008; Del Campo *et al.*, 2011), siendo los resultados proporcionales, dentro de unos límites, a la dosis de aplicación (Al-Humaid y Moftah, 2007; Hüttermann *et al.*, 2009). La dosis de hidrogel recomendada por Del Campo *et al.* (2011) es de 0,1% de polímero respecto a la masa del suelo del hoyo de plantación.

Un factor limitante de esta técnica es que la mejora que produce a nivel de microestación es aprovechada no solo por los brizales objetivo sino también por la vegetación competitiva, lo que puede hacer necesario aumentar los recursos para el control de ésta (Fuentes *et al.*, 2010).

1.3. Técnicas contra la vegetación competitiva

La técnica más habitual contra la vegetación competitiva en plantaciones forestales en Europa es el desbroce químico, aplicando herbicida (Willoughby *et al.*, 2009). Esta práctica se considera el tipo de desbroce más coste-eficiente (George y Brennan, 2002), pero tiene asociado un notable impacto ambiental y una creciente contestación social (Ammer *et al.*, 2011). Como consecuencia, su uso en aplicaciones forestales ha sido prohibido o restringido por parte de diversas administraciones europeas y norteamericanas (Willoughby *et al.*, 2009; Thiffault y Roy, 2011). Una alternativa habitual son los desbroces mecánicos. Éstos pueden aplicarse con maquinaria si el terreno es fácilmente transitable, consiguiéndose un alto rendimiento pero siendo difícilmente aplicables junto al brizal sin causar daños, lo que les resta efectividad. Los desbroces mecánicos también pueden aplicarse en terrenos de mala transitabilidad mediante motodesbrozadora o herramientas manuales, si bien el coste de aplicación aumenta notablemente (Coello y Piqué, 2016). Tanto el desbroce químico como el mecánico tienen un problema añadido, que es la necesidad de aplicarlos de manera recurrente. Una alternativa a los desbroces, especialmente eficiente contra la vegetación herbácea, es la instalación de cubiertas del suelo, también llamadas “mulches” o “acolchados”. Esta técnica consiste en cubrir la superficie del suelo alrededor del brizal con una capa opaca que impide la instalación y proliferación de vegetación competitiva en el entorno de éste (Maggard *et al.*, 2012). Esta técnica permite, por tanto, prevenir o mitigar notablemente el

impacto negativo de la vegetación competitora desde el momento de instalar los brinzales. Además de su efecto contra la vegetación competitora, las cubiertas del suelo pueden tener beneficios adicionales en función de su composición: incremento de la humedad del suelo gracias a la reducción de la evaporación (Barajas-Guzmán y Barradas 2011, McConkey *et al.*, 2012), regulación de la temperatura del suelo (Arentoft *et al.*, 2013) o el aporte de materia orgánica y nutrientes durante su degradación (Merwin *et al.*, 2001; Van Sambeek y Garrett 2004). Numerosas publicaciones han demostrado el interés de las cubiertas del suelo en plantación forestal, habiéndose observado incrementos de la supervivencia, crecimiento y estado hídrico y nutricional del brinal (Pedlar *et al.*, 2006, Chaar *et al.*, 2008; Van Sambeek, 2010; Maggard *et al.*, 2012, Jiménez *et al.*, 2016).

El material más habitual con el que se realizan las cubiertas del suelo es el plástico (lámina de polietileno o tejido de polipropileno), debido a su bajo coste de compra e instalación, su disponibilidad y funcionamiento homogéneo (Arentoft *et al.*, 2013). Sin embargo, este material tiene como principal desventaja su origen no renovable y la necesidad de ser retirado una vez ha cumplido su función, lo cual es una intervención cara y que a menudo no se realiza (Shogren y Rousseau, 2005; Kasirajan y Ngouajio, 2012), lo que da lugar a un riesgo de contaminación de suelos y aguas durante su degradación. Como consecuencia, se están desarrollando en los últimos años cubiertas del suelo realizadas con materiales biodegradables y de origen renovable o reciclado, entre las que destacan los “biofilms”. Este material se emplea de manera creciente en aplicaciones agrícolas pero su uso en plantaciones forestales está limitado por su corta vida útil (Álvarez-Chávez *et al.*, 2012). Otros materiales alternativos al plástico empleados en cubiertas del suelo son los tejidos de fibras vegetales o los derivados del papel (Granatstein y Mullinix, 2008). Además de las cubiertas del suelo en forma de lámina continua, éstas también se pueden realizar mediante partículas apiladas, a base de materiales fácilmente disponibles en la zona de plantación. Entre estos modelos destacan las cubiertas realizadas con paja, astillas o piedras. Las cubiertas de partículas tienen como principal desventaja un funcionamiento heterogéneo en función del tipo y tamaño de partícula y del grosor de la cubierta.

1.4. Objetivos

El objetivo principal de este trabajo es evaluar la efectividad de una serie de técnicas de plantación innovadoras que buscan reducir el impacto negativo de la sequía y la vegetación competidora durante los primeros años de una plantación forestal. Estas técnicas innovadoras buscan mejorar la supervivencia y crecimiento iniciales de los brizales, además de dar lugar a ventajas añadidas en comparación con las técnicas de referencia: i) minimizando la necesidad de aplicar tareas de mantenimiento (funcionamiento autónomo); ii) con un mínimo impacto ambiental considerando su ciclo de vida completo, y iii) con un bajo coste total (compra, transporte, instalación, mantenimiento). Las técnicas innovadoras evaluadas son de dos tipos:

- a) Un prototipo de acondicionador del suelo que incorpora un hidrogel sin poliacrilamida.
- b) Diversos modelos de cubiertas del suelo biodegradables realizadas con materiales de origen renovable o bien reutilizables realizadas con residuos reciclados.

La efectividad de estas técnicas se ha estudiado en comparación con la de otras aplicadas comúnmente para la misma finalidad: acondicionadores con poliacrilamida por un lado, desbroce químico y cubiertas disponibles en el mercado por otro.

2. Resumen de la metodología

2.1. Descripción general de los ensayos

Se han realizado cuatro ensayos de campo independientes, cuyas características se muestran en la Tabla 1.

Tabla 1. Resumen de las características principales de los cuatro ensayos de campo

| | Ensayo 1 | Ensayo 2 | Ensayo 3 | Ensayo 4 |
|-----------------------------|-----------------------------------------------|-----------------------------------------------|-------------------------------------------|----------------------------------------|
| Término municipal | Solsona (L) | Solsona (L) | Mequinenza (Z) | Fontanals de Cerdanya (Gi) |
| Coordenadas | 41°59'12"N 01°30'49"E | 42°00'10"N 01°31'46"E | 41°20'14"N 00°08'34"E | 42°23'09"N 01°55'54"E |
| Altitud media (m) | 670 | 670 | 200 | 1.430 |
| Termotipo | Supramediterráneo | Supramediterráneo | Mesomediterráneo | Montano |
| Ombrotipo | Subhúmedo | Subhúmedo | Semiárido | Húmedo |
| Clima (Köppen) | Csb (templado, verano suave seco) | Csb (templado, verano suave seco) | BS – estepa fría | Cfc – Dfb (templado - continental) |
| Textura; pH del suelo | Franca; 8,2 | Franco-arcillosa; 7,5 | Franco-arenosa; 7,9 | Franco-arenosa; 5,2 |
| Especie arbórea | Nogal híbrido (<i>Juglans x intermedia</i>) | Nogal híbrido (<i>Juglans x intermedia</i>) | Pino carrasco (<i>Pinus halepensis</i>) | Fresno (<i>Fraxinus excelsior</i>) |
| Número de árboles | 360 | 420 | 900 | 450 |
| Tipo de técnicas estudiadas | Cubiertas del suelo, desbroce químico | Cubiertas del suelo | Acondicionadores y cubiertas del suelo | Acondicionadores y cubiertas del suelo |
| Años de estudio | 2011 - 2015 | 2014 | 2014 - 2016 | 2014 - 2016 |

2.2. Diseño experimental

Los ensayos 1 y 2 siguieron un diseño en bloques completos al azar, con 3 y 6 bloques, respectivamente. En el Ensayo 1 se probaron 6 tratamientos (30 brizales por tratamiento) y en el Ensayo 2 se probaron 7 tratamientos (60 brizales por tratamiento).

Los ensayos 3 y 4 siguieron el mismo diseño en bloques completos al azar, con 6 bloques. En cada ensayo se evaluaron 15 tratamientos (30 brizales por tratamiento) resultantes de combinar diferentes acondicionadores del suelo y cubiertas del suelo. En el Ensayo 3 se duplica el diseño en dos terrenos con diferentes orientaciones.

2.3. Tratamientos experimentales

La Tabla 2 muestra el resumen de las técnicas evaluadas en cada ensayo, indicando si se trata de tratamientos de referencia (ya existentes en el mercado en el momento de hacer la experiencia) o innovadores. La Figura 1 muestra el aspecto de cada una de las técnicas aplicadas.

Tabla 2. Técnicas evaluadas en cada ensayo (marcadas con X). R: Técnica de referencia; I: Técnica innovadora.

| | Ensayo 1 | Ensayo 2 | Ensayo 3 | Ensayo 4 |
|--------------------------------------------------------|----------|----------------|--------------|--------------|
| Acondicionadores del suelo | | | | |
| Control | | | X | X |
| R: acondicionador comercial; 40 g;brinzel | | X | X | |
| I: nuevo acondicionador; 20 g;brinzel | | X | X | |
| I: nuevo acondicionador; 40 g;brinzel | | X | X | |
| I: nuevo acondicionador; 80 g;brinzel | | X | X | |
| Total acondicionadores del suelo | - | - | 5 | 5 |
| Técnicas contra la vegetación competitiva | | | | |
| Tamaño de la técnica (cm) | | 100x100 | 80x80 | 40x40 |
| Control | X | X | X | X |
| R: herbicida (aplicación anual de glifosato) | X | X | | |
| R: cubierta plástica: polietileno negro | X | X | X | X |
| R: cubierta comercial de biofilm | | X | X | X |
| I: nueva cubierta de biofilm | X | X | X | X |
| I: cubierta de astilla forestal compostada | X | | | |
| I: cubierta de astilla de poda urbana | X | | | |
| I: cubierta de yute tratado | | X | X | X |
| I: cubierta de goma reciclada | | X | X | X |
| Total técnicas contra la vegetación competitiva | 6 | 7 | 6 | 6 |



Acondicionador del suelo innovador



Árbol control (Ensayo 4)



Desbroce químico (Ensayo 1)



Cubierta de polietileno (Ensayo 2)



Cubierta comercial de biofilm (Ensayo 2)



Nueva cubierta de biofilm (Ensayo 2)



Cubierta de astilla forestal compostada
(Ensayo 1)



Cubierta de astillas de poda urbana
(Ensayo 1)



Cubierta de yute tratado (Ensayo 2)



Cubierta de goma reciclada (Ensayo 3)

Figura 1. Aspecto de cada una de las técnicas evaluadas, y del ensayo al que corresponde cada imagen.

2.4. Variables medidas y análisis estadísticos

Las variables medidas se eligieron por su relevancia como indicadoras del establecimiento inicial de los brizales. Estas variables incluyen medidas directas sobre los brizales y de variables a nivel de microestación. Además se midió la durabilidad de las cubiertas del suelo en dos de los ensayos, con el fin de estimar su vida útil, especialmente, en el caso de las biodegradables. Se muestra a continuación (Tabla 3) las variables medidas en cada ensayo, así como las técnicas estadísticas aplicadas para su análisis.

Tabla 3. Variables medidas en cada ensayo y técnicas estadísticas aplicadas para su análisis.
ED: estadística descriptiva; Chi: Chi-cuadrado de Pearson; M-C: Mantel-Cox; Anova: análisis de varianza; -: variable no medida

| | Ensayo 1 | Ensayo 2 | Ensayo 3 | Ensayo 4 |
|--------------------------------------------------------|----------|----------|----------|----------|
| Medidas directas en los brizales | | | | |
| Supervivencia y estado vegetativo | ED | Chi | M-C | ED |
| Crecimiento anual en diámetro | Anova | Anova | Anova | Anova |
| Crecimiento anual en altura | Anova | Anova | Anova | Anova |
| Biomasa aérea y radical | - | Anova | Anova | - |
| Potencial hídrico antes del amanecer | Anova | - | - | - |
| Potencial hídrico a mediodía | Anova | - | - | Anova |
| Contenido relativo de agua en hoja | - | - | Anova | - |
| Estado nutricional (SPAD) | Anova | - | - | Anova |
| Variables a nivel de microestación | | | | |
| Humedad del suelo | Anova | - | Anova | - |
| Temperatura del suelo | ED | - | - | - |
| Variables específicas de las técnicas empleadas | | | | |
| Durabilidad de las cubiertas | ED | - | - | ED |

Todos los ANOVAs fueron unidireccionales, a excepción de un sub-experimento factorial (combinación de acondicionadores y cubiertas del suelo) de los ensayos 3 y 4. En este caso, se empleó un ANOVA bidireccional para evaluar el efecto de cada tipo de técnica por separado, así como de la interacción entre ambas. Los bloques fueron considerados factores aleatorios en todos los ensayos. En todos los casos se consideró un nivel de significación del 95% ($p < 0,05$). Cuando las diferencias entre tratamientos fueron significativas se hizo un análisis post-hoc, con el método de Tukey (ensayos 1 y 2) o de Duncan (ensayos 3 y 4).

3. Resultados (ensayos)

3.1. Ensayo 1: Can bioplastic or woodchip groundcover replace herbicides or plastic mulching for valuable broadleaf plantations in Mediterranean areas?

Cita: Coello J, Coll L, Piqué M (2017) Can bioplastic or woodchip groundcover replace herbicides or plastic mulching for valuable broadleaf plantations in Mediterranean areas? *New Forests* 48(3): 415-429

El Anexo 1 muestra el artículo correspondiente al Ensayo 1.



Aspecto del Ensayo 1 durante 2013

3.2. Ensayo 2: Use of innovative groundcovers in Mediterranean afforestations: aerial and belowground effects in hybrid walnut

Cita: Vitone A, Coello J, Piqué M, Rovira P (2016) Use of innovative groundcovers in Mediterranean afforestations: aerial and belowground effects in hybrid walnut. Annals of Silvicultural Research 40(2): 134-147

El Anexo 2 muestra el artículo correspondiente al Ensayo 2.



Aspecto del Ensayo 2 durante 2014

3.3. Ensayo 3: Innovative soil conditioners and mulches for forest restoration in semiarid conditions in northeast Spain

Cita: Coello J, Ameztegui A, Rovira P, Fuentes C, Piqué M (2018) Innovative soil conditioners and mulches for forest restoration in semiarid conditions in northeast Spain. Ecological Engineering 118: 52-65

El Anexo 3 muestra el artículo correspondiente al Ensayo 3.



Aspecto del Ensayo 3 (izquierda: solana; derecha: umbría) en 2014

3.4. Ensayo 4: Combining innovative mulches and soil conditioners in mountain afforestation with ash (*Fraxinus excelsior* L.) in the Pyrenees (NE Spain).

Cita: Coello J, Piqué M, Rovira P, Fuentes C, Ameztegui A (2018) Combining innovative mulches and soil conditioners in mountain afforestation with ash (*Fraxinus excelsior* L.) in the Pyrenees (NE Spain). Forest Systems 27(3) – en prensa.

El Anexo 4 muestra el artículo correspondiente al Ensayo 4.



Aspecto del Ensayo 4 en 2014

4. Discusión global

4.1. Interés de los acondicionadores del suelo

Los ensayos 3 y 4, en los que se ha evaluado esta técnica, han mostrado claramente su interés para favorecer el establecimiento inicial de los brizales. A pesar de que estos dos ensayos se han realizado en condiciones muy contrastadas a nivel climático (mesomediterráneo semiárido y montano húmedo, respectivamente) ambos tenían en común un suelo de textura gruesa, franco-arenosa. Por tanto, los suelos de ambos ensayos se caracterizan por una baja capacidad de retención de agua debido a las pérdidas por infiltración y evaporación. En ambos ensayos se ha constatado una mejora generalizada de todas las variables medidas, gracias al acondicionador del suelo: supervivencia, crecimiento y estado hídrico de los brizales (ambos ensayos), estado nutricional de los brizales (Ensayo 4) y humedad del suelo (Ensayo 3).

Los estudios previos realizados con especies leñosas muestran una fuerte disparidad en cuanto a los resultados de la aplicación de esta técnica. Por un lado, los estudios realizados en vivero muestran un efecto netamente positivo del uso de acondicionadores sobre la humedad del suelo y el crecimiento de los árboles (Hüttermann *et al.*, 1999; Arbona *et al.*, 2005; Koupai *et al.*, 2008; Chirino *et al.*, 2011). A nivel de supervivencia, biomasa de raíces y estado hídrico de los árboles los efectos son también predominantemente positivos (Hüttermann *et al.*, 1999, Arbona *et al.*, 2005; Al-Humaid y Moftah, 2007), aunque algunos autores no han encontrado diferencias sobre la supervivencia (Rowe *et al.*, 2005), la biomasa (Chirino *et al.*, 2011) o el estado hídrico (Beniwal *et al.*, 2010).

Los resultados de los estudios de campo son sin embargo más dispares y tan solo se han observado mejoras consistentes en el caso del estado hídrico de los brizales (Clemente *et al.*, 2004; Navarro *et al.*, 2005). En el caso de la supervivencia se han encontrado efectos positivos (Chirino *et al.*, 2011) y nulos (Navarro *et al.*, 2005), mientras que en el caso del crecimiento predominan los estudios en los que esta técnica no ha tenido efecto (Clemente *et al.*, 2004; Barberá *et al.*, 2005; Navarro *et al.*, 2005).

Las causas de estos resultados superiores en el caso de nuestros ensayos en comparación con la mayoría de estudios previos en condiciones de campo pueden estar relacionadas con varios motivos, complementarios entre sí:

- i) Nuestros ensayos se han realizado en terrenos de textura gruesa (franco-arenosa), donde esta técnica es particularmente eficiente (Koupai *et al.*, 2008; Del Campo *et al.*, 2011), mientras que en los estudios realizados en suelos de textura fina los resultados son imperceptibles (Bulíř, 2005; Navarro *et al.*, 2005; Frigola y Nadal, 2013).
- ii) Se han seguido de manera precisa las recomendaciones del fabricante, tanto en lo relativo a su modo de aplicación como a la dosis. En nuestro caso el producto se distribuyó a partes iguales entre el fondo del hoyo de plantación y el suelo empleado para taparlo, mientras que en los estudios en los que se aplica simplemente en superficie, sin mezclarlo con el suelo en profundidad (Hueso-González *et al.*, 2016), los resultados no han sido detectables. En cuanto a la dosis, se aplicó por defecto la cantidad recomendada por el fabricante (40 g/briznizal), a diferencia de estudios en los que se aplicó de manera insuficiente o poco precisa (Navarro *et al.*, 2005; Frigola y Nadal, 2013). La necesidad de una aplicación cuidadosa en cada árbol puede ser por tanto un factor limitante para la adopción de esta técnica a escala operacional.
- iii) Se ha empleado un producto de última generación, desarrollado a principios de 2014, consistente en una mezcla sinérgica de polímero hidroabsorbente con fertilizantes, ácidos húmicos y precursores de la actividad radical, mientras que muchos de los estudios previos utilizan acondicionadores consistentes únicamente en hidrogeles (Clemente *et al.*, 2004; Barberá *et al.*, 2005; Chirino *et al.*, 2011). De esta manera, ha sido posible obtener unos resultados favorables pese a no haber alcanzado la dosis de hidrogel recomendada para suelos arenosos o franco-arenosos por Del Campo *et al.* (2011), fijada en 0,1% en peso del suelo, frente al 0,02% aplicado por defecto en nuestro estudio (40 g/briznizal).

Pese a estos resultados favorables, se ha observado un efecto decreciente de este producto con el tiempo, en línea con los estudios previos (Clemente *et al.*, 2004; Rowe *et al.*, 2005, Holliman *et al.*, 2005; Chirino *et al.*, 2011). Esto sugiere que se trata de una técnica efectiva especialmente durante el primer y, en menor medida, segundo períodos vegetativos. Las causas más probables de este funcionamiento a corto plazo, que han podido coexistir, son dos: por un lado, el agotamiento de los fertilizantes, ácidos húmicos y precursores de la actividad radical, por su absorción por parte de los briznales. Por otro lado, a medida que el sistema radical se desarrolla, las nuevas raíces crecen en un volumen del suelo no mejorado por el acondicionador, por lo que dejan de beneficiarse de su efecto positivo.

En todo caso, los ensayos 3 y 4 han contemplado un seguimiento de tres y dos períodos vegetativos, respectivamente, por lo que estas observaciones deben complementarse con estudios en un plazo más prolongado.

4.2. Dosis y formulación de los acondicionadores

Tanto en el Ensayo 3 (semiárido) como en el 4 (montano) se ha observado que la dosis más coste-eficiente del nuevo acondicionador del suelo para un volumen de aplicación de 30 x 30 x 30 cm es la recomendada por el fabricante: 40 g/brinzal. En el Ensayo 3 esta dosis dio lugar a resultados superiores a los de la dosis más baja (20 g/brinzal) en el caso de la biomasa total, mientras que en el Ensayo 4 se obtuvo un mayor crecimiento en diámetro y altura durante el primer período vegetativo. Además, la dosis de 20 g/brinzal no mejoró los resultados del control (SC-) en algunas medidas de crecimiento anual (ambos ensayos) o del estado nutricional (Ensayo 4), a diferencia de la dosis recomendada. El efecto positivo de incrementar la dosis de acondicionador del suelo ha sido observado en la mayoría de estudios previos (Hüttermann *et al.*, 2009; Del Campo *et al.*, 2010). En nuestros ensayos también hemos observado cómo la dosis más alta (80 g/brinzal) no mejora los resultados de ninguna variable en comparación con la dosis recomendada (40 g/brinzal). Por tanto, este aumento de la dosis solo debería plantearse en caso de aplicarse en volúmenes de suelo de mayores dimensiones.

Respecto a la formulación (nuevo acondicionador sin poliacrilamida en comparación con el acondicionador comercial con poliacrilamida) los resultados de los Ensayos 3 y 4 han sido dispares: en el Ensayo 3 no se observaron diferencias consistentes entre ambos tratamientos, mientras que en el Ensayo 4 se obtuvieron resultados más favorables en el caso del nuevo acondicionador. No ha sido posible discernir la causa de esta diferencia, que probablemente sea debida a la composición de ambos acondicionadores, protegida bajo secreto industrial.

4.3. Interés de las técnicas contra la vegetación competitidora

Todas las técnicas aplicadas contra la vegetación competitidora han tenido un efecto positivo en las estaciones más productivas (ensayos 1 y 2), en las que existe un fuerte desarrollo de la vegetación competitidora y donde se han empleado tratamientos de 100 x 100 cm (Ensayo 1) o de 80 x 80 cm (Ensayo 2). En estos ensayos las técnicas contra la vegetación competitidora (desbroce químico y cubiertas del suelo) han dado lugar a una mejora generalizada de la supervivencia y el crecimiento en diámetro y altura, así como de la biomasa radical (Ensayo 2) y del estado hídrico de los brizales (Ensayo 1), en comparación con el control. Sin embargo, no se detectó ningún efecto sobre el estado nutricional de los brizales (Ensayo 1). A nivel de microestación, en el Ensayo 1 se observó un incremento de la humedad del suelo y un efecto de regulación de la temperatura del suelo en el caso de las cubiertas de astillas.

Sin embargo, los resultados han sido muy diferentes en los Ensayos 3 (semiárido) y 4 (montano), realizados en condiciones poco productivas en las que la vegetación competitidora presenta un desarrollo muy lento y donde se han empleado cubiertas de 40 x 40 cm. En estos casos los efectos positivos de las cubiertas del suelo se han limitado a ligeras mejoras de la supervivencia (Ensayo 4) y a incrementos puntuales del crecimiento (Ensayos 3 y 4) y de la humedad del suelo (Ensayo 3). También se detectaron ligeras mejoras, aunque estadísticamente no significativas, en otros parámetros, como por ejemplo el estado hídrico y nutricional del Ensayo 4. En ambos ensayos el ahoyado realizado tuvo una superficie de 40 x 40 cm, en cuyo centro se instaló el brizal, en un sub-hoyo de 30 x 30 x 30 cm. Esta superficie de suelo removido (40 x 40 cm) permaneció en general libre de vegetación, incluso en los brizales control, durante el conjunto de años de seguimiento de los brizales: 3 años en el Ensayo 3; 2 años en el Ensayo 4. Por tanto, la simple ejecución del ahoyado fue suficiente para evitar el efecto negativo de la vegetación competitidora durante los primeros años (Chaar *et al.*, 2008), debido a la baja productividad de ambas estaciones. Pese a que se esperaba una proliferación poco intensa de la vegetación competitidora en ambas condiciones, ésta fue prácticamente inexistente.

Estos resultados están en línea con estudios previos, en los que el efecto de las cubiertas del suelo sobre el establecimiento inicial de los brizales está ligado a la productividad del terreno y a las dimensiones de las cubiertas. Las cubiertas de dimensiones 80 x 80 cm o superiores, empleadas en condiciones productivas como las de los ensayos 1 y 2 suelen tener un efecto claramente positivo (Paris *et al.*, 1998; Smith *et al.*, 2000; Blanco-García y Lindig-Cisneros, 2005; Paris *et al.*, 2005; Olivera *et al.*, 2014; Jiménez *et al.*, 2014, 2016). Sin embargo, en

aquellos estudios en los que se emplean cubiertas de dimensiones menores (60x60 cm o inferiores, como es el caso de los ensayos 3 y 4), su efecto tiende a ser poco evidente o nulo (Samyn y De Vos, 2002; Navarro *et al.*, 2005; Dostálek *et al.*, 2007; Valdecantos *et al.*, 2009, 2014). Mechergui *et al.* (2018) tampoco observaron un efecto positivo de la utilización de cubiertas del suelo hechas de astillas a pesar de tener unas dimensiones de 100x100 cm, si bien su escaso grosor (3 cm) pudo limitar su eficacia.

En cuanto a las especies, se ha observado cómo el nogal presenta una reacción particularmente favorable al tratamiento de la vegetación competitora (Paris *et al.*, 1998; Paris *et al.*, 2005; Van Sambeek, 2010), mientras que las coníferas (pino carrasco en el Ensayo 3) suelen responder de manera menos apreciable a esta técnica (Siipilehto, 2004; Van Sambeek, 2010; Blanco-García *et al.*, 2011; McConkey *et al.*, 2012).

La ausencia de efecto de las cubiertas del suelo sobre el estado nutricional de los árboles, observado en los Ensayos 1 y 4, está en línea con lo observado por Cregg *et al.* (2009). En el caso del Ensayo 1, realizado en condiciones productivas, este resultado sugiere que la competencia por los nutrientes causada por la vegetación espontánea es poco relevante. En el Ensayo 4, realizado en condiciones limitantes, el escaso desarrollo de la vegetación competitiva ha podido limitar la utilización de los nutrientes del suelo por parte de ésta. En todos los ensayos cabría esperar en los próximos años un posible efecto de la degradación de las cubiertas biodegradables sobre el estado nutricional de los árboles y el suelo.

A nivel de la microestación, el efecto positivo observado en el Ensayo 3 (semiárido) sobre la humedad del suelo a causa de las cubiertas, incluso cuando éstas tienen pequeñas dimensiones, fue también observado en otras zonas secas por Valdecantos *et al.* (2009).

4.4. Evaluación de las diferentes técnicas contra la vegetación competitidora

En cada uno de los ensayos se han empleado diferentes técnicas contra la vegetación competitidora (a excepción de los ensayos 3 y 4, donde sí han coincidido), por lo que no es posible extraer conclusiones generales de cada una de ellas para todas las condiciones estudiadas. Entre las diferentes cubiertas del suelo, la situación predominante en los cuatro ensayos ha sido la ausencia de diferencias significativas entre los diferentes modelos empleados. Esta situación es común en estudios previos, y fue observada por Samyn y De Vos (2002), Abouziena *et al.* (2008), Stafne *et al.* (2009); Maggard *et al.* (2012) y Solomakhin *et al.* (2012). Por tanto, las cubiertas innovadoras desarrolladas por sus ventajas técnicas, sociales y ambientales (realizadas con materiales biodegradables y de origen sostenible, o bien reutilizables de origen reciclado) han dado lugar a resultados similares a los de la cubierta de referencia de polietileno negro, por lo que suponen una alternativa a ésta.

Las principales conclusiones de cada técnica empleada contra la vegetación competitidora son:

i) Herbicida (técnica de referencia): esta técnica ha sido empleada de manera optimizada en los ensayos 1 y 2, aprovechando la brotación tardía del nogal híbrido para retrasar su aplicación hasta el mes de mayo. De esta manera, en vez de conseguirse un suelo desnudo de vegetación alrededor del árbol se genera una “cubierta” de hierba seca, suficientemente densa como para proteger el suelo de la insolación directa e impedir la instalación de nueva vegetación, pero muy permeable al agua de lluvia. Los resultados obtenidos con la aplicación de herbicida han sido contradictorios: en el Ensayo 1 (100 x 100 cm) fue la técnica más favorable para la instalación de los brizales, mejorando los resultados de las cubiertas del suelo en gran parte de las variables, mientras que en el Ensayo 2 (80 x 80 cm) no mejoró los resultados de ninguna cubierta del suelo, siendo de hecho el tratamiento contra la vegetación competitidora que dio lugar a unos resultados más pobres. Este tratamiento solo dio lugar a mejores resultados que el control en el caso del crecimiento diamétrico y de la biomasa de raíces gruesas. El motivo de esta diferencia entre ambos ensayos ha podido estar ligado a las dimensiones de la superficie tratada, que fue un 50% superior en el caso del Ensayo 1 con respecto al 2, en el cual la superficie tratada pudo ser insuficiente. Además, el año 2014, durante el cual se desarrolló el Ensayo 2, fue muy húmedo, lo que pudo haber permitido un nuevo desarrollo de la vegetación competitidora después de la aplicación de herbicida. En las publicaciones previas en las que se compara el efecto de herbicidas y de cubiertas del suelo se han encontrado resultados más favorables para los primeros (Bendfeldt *et al.*, 2001; Ceacero

et al., 2012), para las segundas (Geyer, 2002) o más habitualmente una falta de tendencia clara (Haywood, 2000; Stafne *et al.*, 2009; Atucha *et al.*, 2011; Thakur *et al.*, 2012).

ii) Film plástico (técnica de referencia): esta cubierta se ha empleado como referencia en todos los ensayos, siendo además la técnica empleada por defecto en los subexperimentos de los ensayos 3 y 4 en los que se estudian los efectos de las diferentes dosis y formulaciones del acondicionador del suelo. Las principales ventajas observadas para esta técnica son su durabilidad (superior a la de los modelos biodegradables) y su capacidad para incrementar la temperatura del suelo, lo cual puede permitir prolongar el período vegetativo en estaciones frías. Sin embargo, el uso de esta cubierta está limitado por su origen no renovable y por la necesidad de ser retirada al final de su vida útil.

iii) Cubierta tejida de biofilm verde “Ökolys” (referencia): esta cubierta ha sido empleada en los ensayos 2, 3 y 4, dando lugar a resultados similares a los de las otras cubiertas del suelo. Sus principales ventajas son su permeabilidad (estructura tejida), su biodegradabilidad y su origen sostenible.

iv) Cubiertas de astillas (técnica innovadora): los dos modelos ensayados (astillas forestales compostadas y astillas procedentes de poda urbana) han dado lugar a resultados similares entre sí. La principal particularidad de estas cubiertas es su higroscopidad y efecto aislante: en años secos han tenido un funcionamiento menos favorable que el de las cubiertas de lámina (plástico y biofilm), al dificultar la infiltración del agua procedente de episodios de precipitación débiles. Sin embargo, estas cubiertas han presentado una notable capacidad para reducir la evaporación de agua del suelo y para suavizar las temperaturas extremas, en línea con lo observado por Bendfeldt *et al.* (2001), Siipilehto (2004), Cregg *et al.* (2009), Barajas-Guzmán y Barradas (2011) y Arentoft *et al.* (2013). El funcionamiento de este tipo de cubierta, realizada con materiales que pueden ser localmente abundantes, depende en gran medida de su grosor y del calibre y tipo de astillas, por lo que es una técnica difícil de configurar para su uso en diferentes condiciones climáticas.

v) Biofilm negro (técnica innovadora): se han ensayado dos modelos basados en una composición similar y cuya diferencia principal es la presencia de una estructura semirrígida biodegradable para facilitar su instalación en el caso del modelo empleado en los Ensayos 2, 3 y 4, y que no está presente en el Ensayo 1. El funcionamiento de esta cubierta ha sido en general similar al de la cubierta de polietileno negro, en línea con lo observado por Garlotta (2001). Al igual que la cubierta plástica, el biofilm causó en el Ensayo 1 un incremento de las temperaturas máximas del suelo, en línea con Díaz-Pérez y Batal (2002) y Díaz-Pérez *et al.*

(2005), por lo que puede ser interesante en estaciones limitadas por las bajas temperaturas en las que se pretenda prolongar el período vegetativo. Éste es el caso del Ensayo 4, en el que esta cubierta ha sido la que ha dado lugar a los resultados más favorables. La principal ventaja que presenta esta cubierta en comparación con la plástica es su origen renovable y su biodegradabilidad. La durabilidad de los modelos ensayados es de unos 3 (Ensayo 4) ó 4 (Ensayo 1) períodos vegetativos, por lo que se encuentra en el límite de aceptabilidad para una cubierta del suelo en aplicaciones forestales (Coello y Piqué, 2016).

vi) Cubierta de yute tejido (innovadora): en los ensayos 2 (supramediterráneo subhúmedo) y 3 (semírido) esta cubierta es la que ha dado lugar a resultados más favorables, en línea con McCarthy *et al.* (2007). Las causas más probables de estos buenos resultados en estas condiciones han podido estar relacionadas con su color claro y su estructura tejida. Como resultado, esta cubierta evita el incremento excesivo de la temperatura máxima del suelo y es permeable a episodios de precipitación ligera (Debnath, 2014), dos aspectos favorables en el caso de condiciones de marcada mediterraneidad. Además, se trata de una cubierta biodegradable y fabricada con materias primas renovables (fibra vegetal), por lo que presenta un interesante potencial desde el punto de vista técnico y ambiental. Sin embargo, el principal factor limitante de esta técnica es su durabilidad, estimada (Ensayo 4) en unos 3 años, pese a su tratamiento con resinas para prolongar su durabilidad.

vii)Lámina de goma reciclada (innovadora): esta cubierta ha mostrado un funcionamiento similar al del resto de cubiertas en los Ensayos 2 y 3, pero algo inferior al resto en el Ensayo 4, especialmente en relación a la supervivencia. Se trata de una cubierta basada en un material reciclado, cuyo principal interés es su prolongada durabilidad pero que está limitado por su alta densidad. De esta manera, se trata de una cubierta que puede ser recomendable solo en aplicaciones en las que la accesibilidad y la transitabilidad no son limitantes, como pueden ser los sectores de jardinería y paisajismo.

5. Conclusiones

- a) En plantaciones forestales en condiciones mediterráneas la aplicación de técnicas contra la sequía y/o contra la vegetación competitora permiten mejorar notablemente la fase de establecimiento de los brizales.
- b) Los acondicionadores del suelo en forma de mezclas sinérgicas de polímeros hidroabsorbentes con otros componentes (fertilizantes, precursores de actividad de raíces, ácidos húmicos) son una herramienta coste-eficiente para facilitar el establecimiento de una plantación forestal en suelos de textura gruesa con baja capacidad de retención de agua. El éxito de esta técnica está sujeto a su correcta aplicación y dosificación.
- c) Se ha ensayado con éxito un nuevo acondicionador del suelo libre de poliacrilamida, con resultados similares o superiores a los de las formulaciones comerciales basadas en poliacrilamida, por lo que se espera que el nuevo producto tenga un mayor potencial de aplicación por sus ventajas a nivel ambiental y de aceptación social.
- d) Las cubiertas del suelo son una técnica eficiente para mitigar el efecto negativo de la vegetación competitora en una plantación forestal, reduciendo o evitando la necesidad de aplicar desbroces reiterados. Esta técnica ha dado lugar a unos resultados muy satisfactorios en los ensayos realizados en condiciones de alta productividad, donde se han empleado cubiertas de al menos 80 x 80 cm. En condiciones poco productivas, en las que la vegetación competitora tiene un desarrollo poco vigoroso, la aplicación de esta técnica mediante modelos de menores dimensiones (40 x 40 cm) ha dado lugar a mejoras muy leves en comparación con los brizales control, por lo que no parece una técnica prioritaria.
- e) Existe una gran diversidad de modelos de cubierta del suelo disponibles. Si bien las cubiertas evaluadas en cada Ensayo no han presentado grandes diferencias de resultados globales entre sí, éstas pueden tener un comportamiento diferente en cuanto a la dinámica de la humedad y la temperatura del suelo. Por tanto, es necesario elegir un modelo adaptado a las condiciones de cada plantación. Para una misma superficie, los principales factores que condicionan el comportamiento de las cubiertas son su composición, grosor y color.
- f) Se han ensayado con éxito modelos innovadores de cubiertas del suelo realizados con materiales biodegradables, con resultados muy similares a los materiales plásticos de referencia. Este resultado genera oportunidades para las nuevas cubiertas biodegradables desde el punto de vista técnico (no es necesario que sean retirados) y ambiental (origen renovable, no contaminan en su degradación).

g) No ha sido posible demostrar el interés del uso combinado de acondicionadores del suelo y cubiertas del suelo, ya que la primera de estas técnicas ha sido especialmente favorable en estaciones de baja productividad (ensayos 3 y 4) y la segunda ha tenido un efecto positivo en estaciones de alta productividad (ensayos 1 y 2).

h) Los resultados obtenidos deberían complementarse con estudios futuros que incluyan: i) un seguimiento a más largo plazo, no limitado a la fase de establecimiento; ii) variables fisiológicas adicionales (intercambio gaseoso, conductancia estomática) y iii) variables relacionadas con la microestación (humedad del suelo, principalmente) registradas mediante seguimiento continuo.

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Anexo 1. Artículo correspondiente al Ensayo 1.

1 Can bioplastic or woodchip groundcover replace herbicides or plastic mulching for valuable broadleaf 2 plantations in Mediterranean areas?

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7 Abstract

Weed control is fundamental in plantations of valuable broadleaved species. The most common weeding techniques are repeatedly applied herbicides and removable plastic mulching, both raising environmental concerns. We studied the performance of these techniques on a hybrid walnut plantation, compared with three biodegradable mulch alternatives: a prototype bioplastic film, a layer of composted woodchips and a layer of ramial chips. The durability and effect of the treatments on tree performance (survival, growth, physiological traits) and soil features (moisture and temperature) were evaluated over 4 years. Herbicide yielded the best results, while all the mulching treatments provided better results than controls for nearly all the variables. The performance of plastic and bioplastic films was similar, suggesting that the latter could replace plastic mulching. The performance of the two chip mulches was similar and slightly below that of the films, probably because of the excessive thickness of the former (13–14 cm). In summary, biodegradable mulches showed high effectiveness in controlling weeds and so could offer an alternative to herbicide application and plastic mulching when these are contra-indicated technically (accessibility, repeatability), economically (labour cost), legally or environmentally.

Keywords: afforestation; biodegradable mulch; herbicide; *Juglans*; restoration; weed competition

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32 **Introduction**

33 In Mediterranean conditions, the primary limiting factor for tree growth is water shortage during summer
34 drought (Villar-Salvador et al. 2012) which is expected to worsen in the future (Vallejo et al. 2012, Dumroese et
35 al. 2015), and whose negative effect can be exacerbated by a poor soil preparation (Löf et al. 2012) and by
36 weeds. The competition effect of unwanted vegetation is especially intense on highly fertile sites such as former
37 agricultural fields (Kogan and Alister 2010; Olivera et al. 2014), and is particularly harmful to young seedlings
38 with underdeveloped root systems and insufficient leaf area to shade and control weed development unaided
39 (Coll et al. 2003; Devine et al. 2007). The use of herbicides, especially glyphosate, is the most common weed
40 control technique applied in forest plantations in Europe (Willoughby et al. 2009) and most temperate areas
41 worldwide, because of its efficacy against a wide range of weed types (Kogan and Alister 2010). However,
42 growing social and environmental concern about herbicides is curbing their use (Ammer et al. 2011). They are
43 banned in public forests in Quebec (Thiffault and Roy 2011) and in a broad range of forest areas in various
44 European countries, for example Germany, Denmark, Czech Republic and Slovakia (Willoughby et al. 2009).
45 Also, the need for repeated application, at least once a year, and during one particular stage of weed
46 development, limits herbicide use in plantations under low intensity management schemes, when minimizing
47 invested resources is fundamental. Alternatives or supplements to herbicide application include the use of
48 planting strategies to reduce water or nutrient stress from competing vegetation such as the use of nursery
49 nutrient loading or direct application of controlled-release fertilizer to the root zone (Jacobs et al. 2005; Uscola
50 et al. 2015, Schott et al. 2016). Another alternative to repeated, time-consuming chemical or mechanical
51 weeding in tree plantations is one-time application of groundcover or “mulching” (Chalker-Scott 2007). This
52 technique consists of covering the ground around the tree to prevent the germination and growth of weeds
53 (Maggard et al. 2012), being a physical barrier that stops light reaching the soil (Bond and Grundy 2001). The
54 most significant benefit of mulching is increased soil water content, especially during the driest periods
55 (Maggard et al. 2012; McConkey et al. 2012), through preventing water transpiration by weeds and reducing
56 soil water evaporation (Percival et al. 2009; Kumar and Dey 2010; Barajas-Guzmán and Barradas 2011;
57 Zegada-Lizarazu and Berliner 2011). Other reported benefits are extreme temperature buffering (Cregg et al.
58 2009; Barajas-Guzmán and Barradas 2011; Arentoft et al. 2013) and improvement of soil physical properties
59 (Chalker-Scott 2007), which helps trees regulate root respiration and favours water and nutrient uptake (Dodd et
60 al. 2000). Organic mulches may increase soil nutrient and organic matter content during their decomposition
61 (Merwin et al. 2001; Van Sambeek and Garrett 2004). The resulting positive effect of mulching on tree survival
62 and growth is cumulative and perceptible over subsequent decades (George and Brennan 2002; Pedlar et al.
63 2006). To date, most studies on the use of this technique and its effects on vegetation and soil have been
64 conducted in temperate conditions. In areas limited by water, such as prevail in Mediterranean sites, the efficacy
65 of this technique is still unproven.

66 The most widespread mulching material is black polyethylene film (Barajas-Guzmán et al. 2006; Arentoft et al.
67 2013), a low-cost homogeneous material with proven positive effects on forest plantations (Green et al. 2003).
68 Its use is, however, limited by concerns about using long-lifespan plastics in the open air, by its unsightliness,
69 and especially by its high cost of removal and disposal (Shogren and Rousseau 2005). As a result, a wide range
70 of biodegradable alternatives are being developed, including bio-based plastics (Álvarez-Chávez et al. 2012),

71 notably polylactic acid (PLA) (Finkenstadt and Tisserat 2010) and polyhydroxyalkanoate (PHA). These
72 materials show homogeneous behaviour but have not yet been widely evaluated in outdoor conditions for forest
73 restoration purposes. Other biodegradable mulches used in tree plantations include those based on locally
74 abundant (often waste) organic materials, such as straw, woodchips and paper.

75 The aim of this work was to evaluate, on a 5-year basis, whether groundcover based on renewable,
76 biodegradable materials could offer a suitable alternative to herbicide application or plastic-based mulches on
77 valuable broadleaf plantations in Mediterranean conditions. Such plantations are being increasingly considered
78 as an alternative use, with both economic and environmental advantages, for agricultural land facing
79 abandonment (Aletà et al. 2003; Coello et al. 2009) in Mediterranean areas.

80 Our hypothesis was that these novel mulches would yield technical outcomes similar to those of plastic
81 mulching for tree performance and micro-site features, while being more environmentally friendly and not
82 needing removal. They could also usefully replace herbicide application, being cost-saving (less labour-
83 intensive) and ecologically and socially more acceptable.

84

85 **Methods**

86 *Study area and experimental design*

87 The study was conducted in Solsona, NE Spain (41°59'N; 1°31'E), in a flat, homogeneous, former agricultural
88 field, located at an elevation of 670 m a.s.l., and farmed until the year of planting for winter cereal production
89 (wheat, barley and oats). The study area had a Mediterranean continental sub-humid climate (Martín-Vide 1992)
90 or Mediterranean continental Csb (Temperate, dry mild summer) in the Köppen classification. Mean annual
91 temperature is 12.0 °C and mean annual and summer precipitation are 670 mm and 171 mm, respectively
92 (Ninyerola et al. 2005). The analysis of soil samples taken at three different points at depth 5-30 cm revealed a
93 loamy texture (21% clay, 45% silt, 34% sand) and pH 8.2. Initial soil organic matter content was 2.3% and
94 active limestone was 5.2%. The main weed species were first *Avena fatua* and second *Lactuca serriola*.

95 One experimental plantation was set up in March 2011 with 1-year-old hybrid walnut (*Juglans × intermedia*)
96 MJ-209xRa, 40–60 cm high, bare-rooted, planted on a 4 × 4 m frame. Soil preparation consisted in deep (50
97 cm), crossed sub-soiling with a 150 HP tractor with chisel, and pits were opened manually just before tree
98 establishment. Six different vegetation control conditions were then applied: (i) chemical weeding (glyphosate,
99 22.5 cm³/tree at 1.25%) applied yearly in May with a backpack sprayer (HERBICIDE), (ii) commercial black
100 polyethylene film, treated against UV radiation, 80 µ thick (PLASTIC), (iii) prototype black PHA
101 (polyhydroxyalkanoate) film, 100% biodegradable, 80 µ thick (BIOFILM), (iv) a layer of woodchips made with
102 woody debris from pine forest harvesting operations, composted for 8 months, size 15/35 mm, layer thickness
103 13/14 cm (WOODCHIPS), (v) a layer of fresh ramial woodchips (branches and twigs from urban pruning), size
104 15/35 mm, layer thickness 13/14 cm (RAMIALCHIPS), and (vi) no treatment (CONTROL). Each weeding
105 treatment was applied on 100 × 100 cm of soil (centred on the tree). No artificial watering or fertilization was
106 applied to the experimental area.

107 These treatments were deployed in a randomized complete block design with subsamples. Each of the five
108 blocks consisted on six randomly distributed plots (one per treatment) with 12 trees each (192 m²), for a total of
109 72 trees per block (1,152 m²) and 360 experimental trees in all (5,760 m²).

110 *Weather and soil variables*

111 Daily temperature and precipitation data were obtained during the study period from a nearby weather station
 112 belonging to the Catalan Meteorological Service, located less than 1 km away from the study site at a similar
 113 altitude. The first two vegetative periods (lasting normally from April-May to October), namely 2011 and 2012,
 114 were notably drier and warmer than the historical average, especially during summer, with precipitation 60%
 115 and 70% lower than the historical average. Precipitation was not only low but also unevenly distributed, with
 116 few episodes of significant rainfall. However, 2013, 2014 and 2015 were much wetter, with summer
 117 precipitation close to the historical average (2013 and 2015) and 36% higher (2014) and with a fairly regular
 118 distribution of rainfall episodes (Table 1).

119 **Table 1** Weather summary 2011–2015, summarizing the variables most relevant to tree growth. Source: Servei
 120 Meteorològic de Catalunya. Summer extends from 21 June to 20 September (92 days).

| Year | Mean daily | Mean maximum | Annual | Summer | Number of summer days | |
|-----------|----------------|-------------------|--------------|---------------|-----------------------|--------|
| | temperature in | daily temperature | precipitatio | precipitation | with precipitation... | |
| | summer (°C) | in summer (°C) | n (mm) | (mm) | >10 mm | >25 mm |
| Reference | 20.3 | 27.2 | 670 | 171 | n.a. | n.a. |
| 2011 | 20.9 | 28.8 | 568 | 68 | 2 | 0 |
| 2012 | 21.8 | 30.0 | 464 | 52 | 1 | 1 |
| 2013 | 20.5 | 29.3 | 637 | 173 | 6 | 3 |
| 2014 | 20.2 | 27.9 | 713 | 233 | 7 | 5 |
| 2015 | 21.8 | 30.2 | 520 | 195 | 5 | 2 |

121
 122 Soil volumetric water content (l water per 1 soil) at depth 5–20 cm was estimated gravimetrically on two
 123 different dates: June 2012 and August 2014. Each sampling campaign was conducted after at least 2 weeks
 124 without rain, and consisted of the extraction of four soil samples per treatment in 3 blocks (144 in all). Sampling
 125 points were located 30 cm away from the tree stem at aspect 225° (SW); approximately 150 g of soil was
 126 collected with a soil auger. Samples were kept in tared, zip-closed plastic bags, and weighed within 3 h at the
 127 laboratory on a precision scale (0.1 g) to obtain fresh weight, after subtracting the bag weight. Dry weight was
 128 obtained after leaving the bags fully open at 65 °C for 96 h, using the same procedure.

129 Soil temperature at depth 7.5 cm was recorded continuously from the time of tree planting using thermometers
 130 with built-in dataloggers. There were three thermometers per treatment (one per block, in 3 blocks), located 30
 131 cm away from the tree in aspect 225° (SW), each tied to a short rope for easy retrieval. The results for soil
 132 temperature are shown for three key periods: a representative flushing period (1-20 May 2012), the warmest
 133 spell (11–26 August 2011) and the coldest spell (2-23 February 2012) after the time of planting.

134 *Tree measurements and mulch durability*

135 Seedling mortality was monitored at the end of each vegetative period (2011-15), together with any signs of
 136 vegetative problems (death of the apical shoot or presence of basal sprouting). Plant basal diameter and total
 137 height were recorded at the time of planting, and then at the end of each growing season using a digital calliper
 138 and a measuring tape, respectively. The diameter and height growth of a living tree during a vegetative period
 139 was calculated as the difference between two consecutive measurements. Tree height growth was considered to

140 be zero for a given year when the apical shoot was dead, but the height of the highest living bud was measured
141 to have the initial size for the subsequent year.

142 Physiological variables were measured within the growing seasons. During July 2012, predawn (07:00 solar
143 time) and midday (12:00 solar time) leaf water potential were measured with a pressure chamber (Solfranc
144 technologies, Vila-Seca, Spain) using ten leaves per treatment collected from different trees from 3 blocks. In
145 July and August 2014 midday leaf water potential was measured likewise. In all cases the measuring day was
146 chosen after at least two weeks without rain. Finally, leaf chlorophyll content was estimated using a Minolta
147 SPAD-502 instrument (Minolta Camera Co. Osaka, Japan) in both July 2012 and July 2014 on fully elongated
148 leaves exposed to direct sunlight. For each treatment, 10 trees from 3 blocks were sampled, and the SPAD (Soil
149 Plant Analysis Development) value, a relative indicator of leaf greenness (Djumaeva et al. 2012) was obtained
150 for each tree as the average of three fully elongated, sun-exposed leaves.

151 Forty months after the start of the experiment (June 2014), the durability of the four mulching treatments was
152 assessed by visual estimation of the percentage (rounded to tens) of mulched area free of weeds (chip mulches)
153 or free of weeds and also physically intact (film mulches). Mulch status was rated effective (showing at least
154 80% intact surface), partially damaged (40–70% intact surface) or ineffective (30% or less intact surface).

155 *Statistical analysis*

156 The data related to soil moisture, tree growth, tree water status and leaf chlorophyll were analysed
157 independently for each measuring date. We used an analysis of variance (ANOVA), considering both treatment
158 and block as fixed factors, and following the model:

$$159 \quad Y_i = \mu + \alpha_i + \beta_j + e_{ij} + \delta_{ijk};$$

160 where Y is the dependent variable; μ is the population mean for all treatments; α_i is the treatment effect; β_j is the
161 block effect; e_{ij} is plot error and δ_{ijk} is subsample error.

162 Normality of residuals was confirmed with the Kolmogorov – Smirnov test. To meet this condition we used a
163 square root transformation of the diameter growth values and the zero values of height growth were transformed
164 to 0.0000001 (Kilmartin & Peterson, 1972). Differences between treatments were examined by a post hoc
165 Tukey test with the significance threshold set at $p < 0.05$. All these analyses were performed with SPSS v.19.0
166 software (IBM SPSS Statistics for Windows, Armonk, NY, USA 2010). The rest of variables (soil temperature,
167 tree survival and mulch durability) were analysed with descriptive statistics.

168 **Results**

169 *Plant survival and growth*

170 In general, mortality rates were low, less than 6% for all the treatments together along the five growing seasons.
171 Most mortality occurred in unweeded plants (CONTROL), which showed an overall mortality of 33% at the end
172 of 2012. This year was the driest and warmest, showing 94% of all casualties; there was no mortality thereafter.
173 Vegetative problems (loss of apical shoot or basal sprouting) were especially frequent in the CONTROL (25%
174 on average in the five vegetative periods) and chip groundcover (RAMIALCHIPS – 26% and WOODCHIPS –
175 23%) plots, with the other treatments below 15%. Most of the vegetative problems (90%) arose during 2011 to
176 2013, affecting 26% of the trees considering all treatments, this figure falling to 6% in 2014 and 4% in 2015
177 (Table 2).

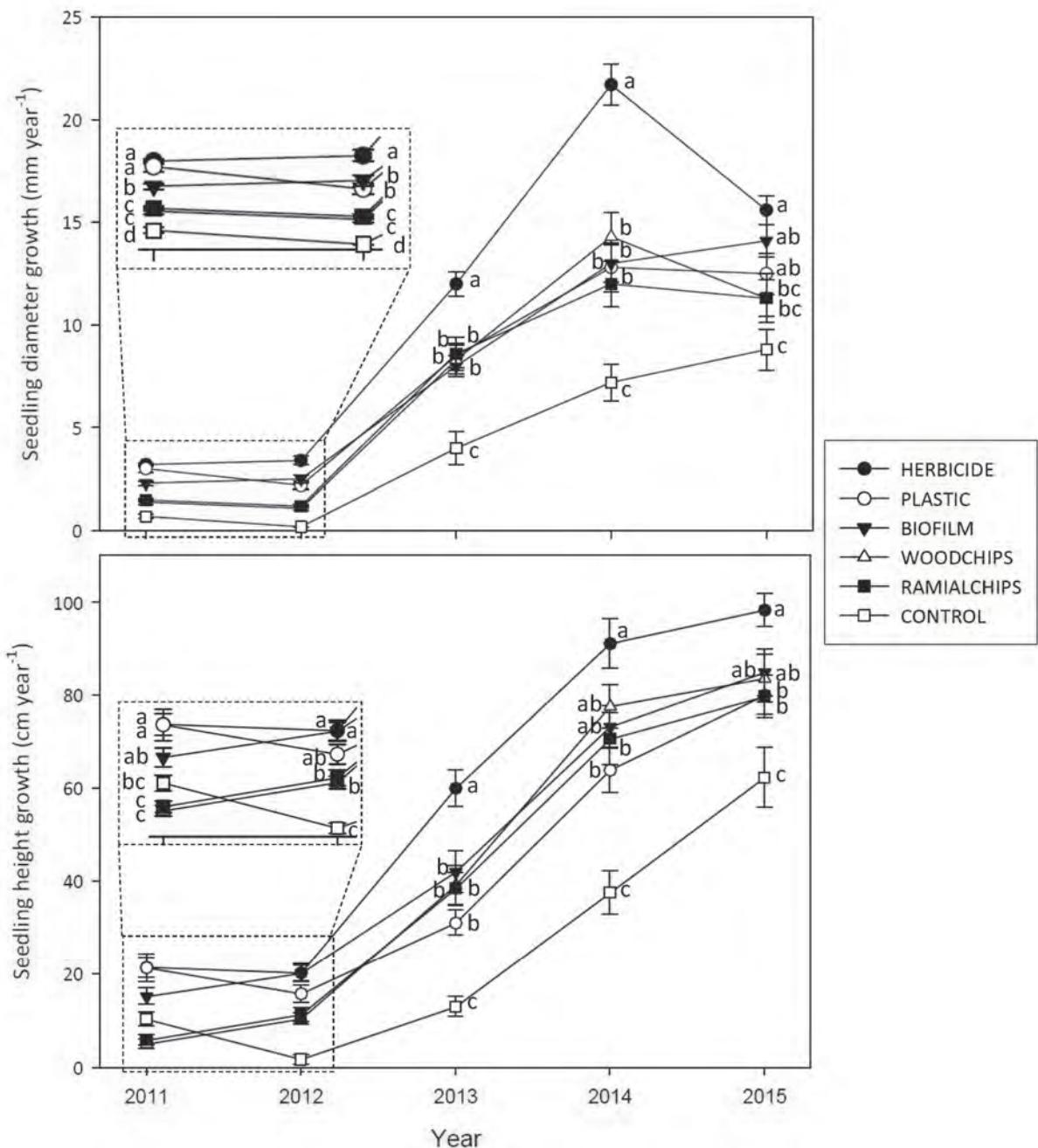
178 **Table 2** Number of hybrid walnut trees suffering from vegetative problems and dying during the first five
179 growing seasons, for each weeding technique. Mortality is cumulative, while the occurrence of vegetative
180 problems in a tree could vary from year to year.

| | Alive, with vegetative problems | | | | | Dead | | | | |
|-------------|---------------------------------|------|------|------|------|------|------|------|------|------|
| | 2011 | 2012 | 2013 | 2014 | 2015 | 2011 | 2012 | 2013 | 2014 | 2015 |
| HERBICIDE | 16 | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| PLASTIC | 17 | 6 | 8 | 2 | 3 | 0 | 0 | 0 | 0 | 0 |
| BIOFILM | 18 | 5 | 7 | 4 | 3 | 2 | 2 | 2 | 2 | 2 |
| WOODCHIPS | 21 | 17 | 24 | 4 | 2 | 0 | 3 | 3 | 3 | 3 |
| RAMIALCHIPS | 14 | 24 | 25 | 10 | 2 | 0 | 5 | 5 | 5 | 5 |
| CONTROL | 26 | 27 | 18 | 2 | 2 | 0 | 33 | 33 | 33 | 33 |

181
182 Throughout the study period there was a consistent and remarkable treatment effect ($p<0.001$ in all cases) on
183 annual tree diameter and height growth. The HERBICIDE plots gave predominantly the best results of all the
184 treatments in terms of seedling growth, while CONTROL gave the poorest results (Figure 1). Mulching
185 treatments provided intermediate outcomes, with films (PLASTIC and BIOFILM) resulting in higher growth
186 rates than chip layers (WOODCHIPS, RAMIALCHIPS) during the driest years (2011 and 2012).

187 Through 2013 - 2015 the average annual diameter growth was 15.7 mm for HERBICIDE, 10.9 mm for the
188 mulches taken together and 6.5 for CONTROL. Annual height growth values in the last three growing seasons
189 showed similar trends, with HERBICIDE at 83 cm, followed by 65 cm for mulches and 39 cm for CONTROL.

190



191

192 **Fig. 1** Diameter and height growth time course of hybrid walnut during the first five growing seasons. Growth
 193 was calculated as the difference between the basal diameter (mm) or total height (cm) at the end and at the
 194 beginning of each growing season. Significant differences ($p < 0.05$) between treatments found each year are
 195 indicated by different letters, and were grouped according to Tukey test.

196

197

198

199 *Plant and soil water status and chlorophyll content*

200 During the driest year of the study period (2012), HERBICIDE provided the highest levels of soil moisture and
201 the least negative soil and plant water potential values, while CONTROL gave the poorest results for all these
202 variables (Table 3). The only significant differences in the effects of mulching treatments on plant and soil water
203 potential were obtained in 2012, when in June BIOFILM plots showed more favourable pre-dawn leaf water
204 potential values (a proxy of soil water status) than the other mulches, and in July, a more favourable midday leaf
205 water potential than RAMIALCHIPS. In 2014, a particularly wet year, both HERBICIDE and RAMIALCHIPS
206 resulted in better plant water status than CONTROL, while the other treatments showed no significant
207 difference. Finally, soil moisture produced minor differences between mulching treatments, consisting in
208 prevalently higher values in the case of chip mulches compared with film mulches (Table 3). The measurements
209 of leaf chlorophyll content made in July 2012 and July 2014 showed no significant difference between
210 treatments. In 2012, HERBICIDE showed the highest value (35.4 ± 0.9) followed by CONTROL (34.5 ± 1.05),
211 while the average value of mulch treatments ranged between 33.1 and 33.6. Trees showed slightly higher SPAD
212 values in 2014, led by BIOFILM (41.3 ± 1.9), HERBICIDE (40.7 ± 2.2) and CONTROL (40.2 ± 1.7), while the rest
213 of mulch treatments had averages ranging between 38.9 and 39.2.

214

215 **Table 3** Plant and soil water status, expressed as leaf water potential and volumetric soil water content,
216 respectively. Each measurement of leaf water potential refers to 60 trees (10 per treatment). Each soil moisture
217 monitoring refers to 144 trees (24 per treatment). F-values and the level of significance of each analysis are
218 provided.

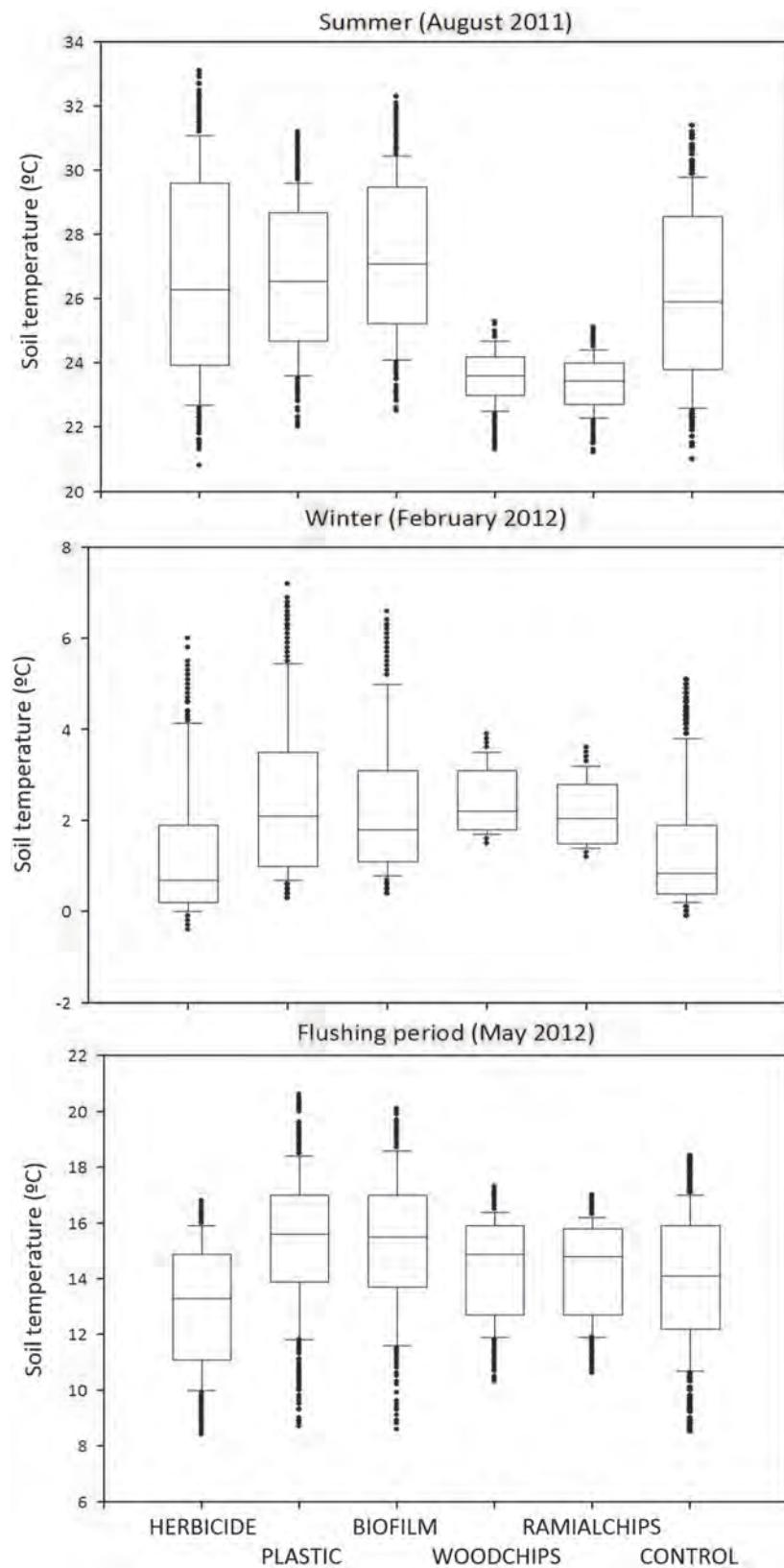
| | Leaf water potential (bar) | | | | Soil moisture (% volume) | |
|-------------|----------------------------|---------------|---------------|---------------|--------------------------|------------|
| | Predawn ψ | Midday ψ | Midday ψ | Midday ψ | June GS2 | August GS4 |
| | June GS2 | June GS2 | July GS4 | August GS4 | | |
| F-value | 81.8** | 11.9** | 4.7* | 2.35* | 25.9** | 2.87* |
| HERBICIDE | -2.56a | -13.4a | -12.7a | -15.0a | 16.3a | 11.7ab |
| PLASTIC | -5.61c | -16.1b | -13.6ab | -16.4ab | 10.7bc | 10.0b |
| BIOFILM | -3.81b | -15.9b | -12.9a | -16.0ab | 10.4c | 11.3ab |
| WOODCHIPS | -6.03c | -16.3b | -13.6ab | -16.0ab | 12.1b | 11.4ab |
| RAMIALCHIPS | -5.13c | -15.7b | -14.7bc | -15.5a | 12.1b | 11.9a |
| CONTROL | -12.3d | -18.1c | -16.3c | -18.0b | 8.7d | 10.1b |

219 * significant at 0.05 level ** significant at 0.01 level

220

221 *Soil temperature*

222 Mulches had a remarkable buffer effect on soil temperature, especially chips (Figure 2). For example, during a
223 representative flushing period (May 2012) the minimum soil temperatures were 2 °C higher in chip mulch plots
224 than in the other treatments. During the warmest period of the study (August 2011) the buffering effect of chip
225 layer mulches was even stronger, with mean and average maximum temperatures respectively 4 °C and 6 °C
226 lower than the average for the other treatments. Finally, during the coldest period of the series (February 2012)
227 the effects of chip mulches were less noteworthy, with minimum soil temperatures 1 °C higher than in the case
228 of film mulches, and 2 °C higher than for HERBICIDE and CONTROL, which were the only treatments in
229 which soil temperatures fell below 0 °C. The most noticeable effect of film mulches was the increase, by 2–4
230 °C, in the maximum soil temperatures during the flushing period (May 2012) compared with CONTROL and
231 chip layer mulches. Finally, HERBICIDE and CONTROL in general showed similar soil temperature trends,
232 with slightly higher maximum temperatures during the flushing period for CONTROL, and during summer and
233 winter for HERBICIDE.

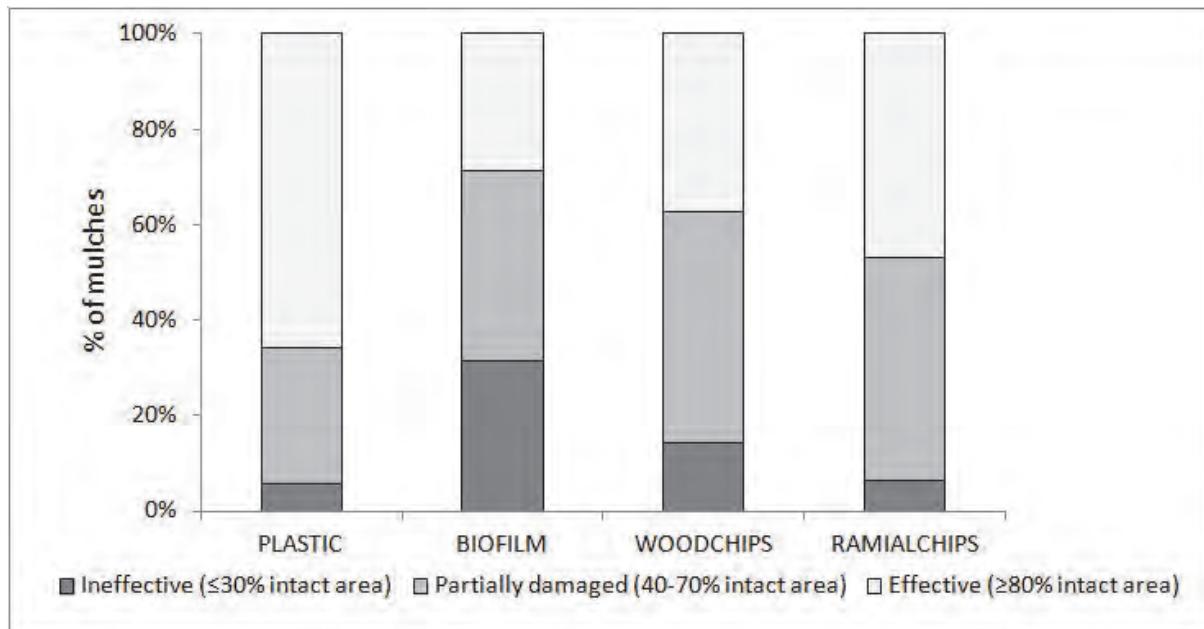


234

235 **Fig. 2** Distribution of soil temperatures at depth 7.5 cm during the warmest spell (16 days in August 2011), the
 236 coldest spell (23 days in February 2012), and a representative flushing period (21 days in May 2012). The box
 237 length represents the interquartile range (Q1 to Q3) while the horizontal line inside the box indicates the median.

238 *Durability of mulches*

239 The durability assessment of mulching performed after 40 months (Figure 3) gave PLASTIC the highest
240 proportion of effective units: two thirds of polyethylene mulches kept at least 80% of their surface being
241 effective, whereas only 6% were rated ineffective (30% or less of their surface being effective). By contrast,
242 approximately one third of BIOFILM mulches were found effective at the evaluation time and a similar
243 proportion were ineffective. The results for chip mulches fell between the two film mulches, with
244 RAMIALCHIPS showing slightly higher durability than WOODCHIPS, with 47% and 37% of effective units
245 and 6% and 14% of ineffective units.



246
247 **Fig. 3** Durability of mulching treatments after 40 months (March 2011 to June 2014), based on visual evaluation
248 (percentage of intact area, rounded to tens).

249

250

251 **Discussion**

252 Hybrid walnut is characterised by late flushing, second half of May in our study area, which is four to five
253 weeks after most weed emergence. This property allowed herbicide application to be postponed until early-to-
254 mid-May. Chemical weeding was therefore applied on grown weeds (around 50–70 cm high on average), which
255 left not a bare soil but a dry grass cover that was (i) free from transpiring vegetation, (ii) dense enough to
256 impede new weed proliferation and stop sunlight reaching the soil, thus mitigating soil water evaporation, and
257 (iii) highly permeable to water infiltration. These three features are especially beneficial during summers with
258 infrequent precipitation distributed in low-volume episodes (typical in Mediterranean climates, and most
259 common in 2011 and 2012 in our case) and might in part explain the outstanding results of the HERBICIDE
260 treatment found in this study.

261 Mulching provided better results than CONTROL for most of the variables and measuring dates, as reported in
262 most previous studies (Johansson et al. 2006; Abouziena et al. 2008; Maggard et al. 2012). Among the different
263 mulching treatments, the relatively poor results of chip mulches during the dry years (2011 and 2012) might be
264 linked to the thickness of the layer used (13–14 cm). This thickness was chosen based on previous studies
265 (Granatstein and Mullinix 2008; Percival et al. 2009) carried out in wetter areas. Mulches made of chips and
266 other organic materials need to be moistened adequately before they become permeable to water and let it
267 through to the soil. In particularly dry summers with precipitation in small volume episodes as those which
268 occurred in our study in 2011 - 2012, excessively thick organic mulches can prevent water reaching the soil
269 (Gilman and Grabosky 2004). Film mulches (PLASTIC and BIOFILM) may also have kept water from reaching
270 the soil during the low-volume rainfalls of 2011 - 2012. The two mulches yielded relatively similar results
271 between them for both plant and soil variables. The similar mechanical properties of biofilms and plastic were
272 also reported by Garlotta (2001). In general terms, black biofilm groundcover can be considered as a useful
273 substitute for black polyethylene film: it is made from renewable raw materials and is biodegradable, so there is
274 no disposal cost at the end of its service life. PHA belongs to the group of preferred bioplastics as regards
275 environmental, health and safety impacts (Álvarez-Chávez et al. 2012) and highest production (Iles and Martin
276 2012). In addition, the growing demand for bioplastics in multiple applications is expected to bring down prices
277 in the near future (Iles and Martin 2012). The choice of one formulation (plastic or bioplastic) over another
278 could depend on differences in cost of purchase (lower for polyethylene) and removal (nil for biodegradable
279 mulches).

280 Van Sambeek (2010) analysed 50 papers for the effect in terms of growth and fruit production of different
281 weeding techniques on walnut, expressed as a relative response. The herbicide/control response ratio was 178,
282 i.e. trees treated with herbicide yielded 78% more than those that were unmanaged. The figure was 267 for
283 synthetic mulches (mostly polyethylene) and 265 for organic ones. In our study the average growth response
284 (diameter and height) of HERBICIDE relative to CONTROL (unmanaged) was 272, much higher than the
285 above reported response. However, mulches resulted in poorer relative responses than in that study, with
286 PLASTIC giving a value of 196 and chip mulches averaging 180. These divergences could arise from (i) the
287 delayed application of the herbicide treatment adapted to a late-flushing tree species, and (ii) the occurrence in
288 our study of particularly dry summers with very infrequent rainfall events in the two first years after planting,
289 when (as stated above) mulches may have hindered water infiltration into the soil.

The trial presented mortality rates below 6% considering all treatments, indicating the suitability of the species for the site, despite the unusually dry 2011 and 2012. While mortality in 2011 was negligible (one seedling in all), it rose to 15 further seedlings at the end of 2012, probably as a consequence of the poor root development in 2011 (Watson 2005) and the depletion of reserves after two consecutive harsh years. The CONTROL treatment accounted for 75% of the dead seedlings of the study, highlighting the effect of weeding on survival (Green et al. 2003; Van Sambeek and Garrett 2004). Similarly, Green et al. (2003) and Chaar et al. (2008) found that the positive effect of weeding with respect to unweeded trees was especially noticeable in the second year after plantation. Vegetative problems, especially loss of apical shoots and symptoms such as basal sprout emergence, were especially frequent (one third of the trees) during the first vegetative period, probably owing to the particular harsh conditions in 2011 and post-transplant shock effects (Oliet et al. 2013). However, during the following years the number of trees with vegetative problems diminished, indicating sound acclimatisation of the trees to the site conditions. We found a clear difference in the time course of growth in the plots under weed management (HERBICIDE and mulching) and the CONTROL plots between 2011 and 2012. Whereas the weeded trees kept a relatively similar aboveground growth rate during both years, CONTROL tree growth slowed dramatically in 2012, as did survival rate, in line with the results of Coll et al. (2007) in hybrid poplar plantations established in forest sites. The ranking of treatment performance (HERBICIDE > mulching > CONTROL) was consistent throughout the period (Figure 1).

With regard to plant and soil water status, HERBICIDE yielded better results than mulching during the driest year of the study period (2012), probably owing to the sparse rainfall episodes in that summer, which may have limited the amount of water reaching the soil in mulched trees. A similar situation was observed by Ceacero et al. (2012) in a dry year in southern Spain. However, during wet years (2014) only HERBICIDE only provided better plant water status than CONTROL, while not being consistently better than any of the mulch models. Similarly, in areas with moderate-to-high water availability, such as Central USA (Maggard et al. 2012) or in irrigated orchards in NW India (Thakur et al. 2012) and SW Russia (Solomakhin et al. 2012), mulching did not result in lower soil water content than after herbicide application. On the other hand, mulching increased soil moisture compared with CONTROL during warm dry years (2012) as a result of buffered maximum temperatures, lower evaporation and decreased transpiration due to weed suppression (Barajas-Guzmán et al. 2006; Zhang et al. 2009; Maggard et al. 2012; McConkey et al. 2012). The lack of effect of any treatment on the SPAD measurements indicates that weed competition did not affect the chlorophyll content of walnut leaves, a variable closely related to nutritional status, especially with regard to nitrogen. This was also found by Cregg et al. (2009) after 2 years with both polyethylene and chip mulches. In addition, the process of degradation of chip mulches did not lead to either an increase or a decrease in nitrogen content at plant level. Nitrogen release may be expected in subsequent years, especially with RAMIALCHIPS, composed mostly of thin branches of both broadleaf and conifer species, while nitrogen shortage is more likely with WOODCHIPS, rich in pine bark. Finally, the buffering effect of the mulches on extreme temperatures was especially noticeable in the case of chip mulches (WOODCHIPS, RAMIALCHIPS) in all seasons, in line with Cregg et al. (2009), Barajas-Guzmán and Barradas (2011) and Arentoft et al. (2013). By contrast, film mulches (PLASTIC, BIOFILM) did not buffer, but instead increased maximal summer soil temperatures (Díaz-Pérez and Batal 2002; Díaz-Pérez et al. 2005).

328 The effectiveness of mulching techniques was evaluated after 40 months, a period long enough to let trees
329 develop a root system and/or sufficient leaf area to shade and mitigate weed development unaided (Coll et al.
330 2003; Devine et al. 2007), and thus adequate to estimate whether or not the mulches had reached their expected
331 service life (Coello and Piqué 2016). PLASTIC mulches were found to be especially durable, with 66% still
332 effective (80% or more intact surface), as in Haywood (2000), who observed 70% of plastic mulches free of
333 weeds after 5 years. RAMIALCHIPS and WOODCHIPS were close to the end of their service life in view of
334 the incipient weed development on their areas, with roughly 50% and 40% of effective units, respectively.
335 Finally, BIOFILM was approaching the end of acceptable service life, given that approximately one third of the
336 mulches fell into each of the three damage categories (effective, partially damaged or ineffective). This
337 particular model of a pre-commercial prototype probably needs slight modification in composition or thickness
338 to offer the desirable durability for afforestation in areas subject to higher sun and heat radiation.
339 These results correspond to a single trial installed in a homogeneous, flat field, representative of Mediterranean
340 continental sub-humid conditions. However, they are to be complemented by further research in additional trials
341 and pedoclimatic conditions in order to have more consistent results on the efficacy of the treatments tested.
342

343 Conclusion

344 Our study shows that on highly productive Mediterranean continental sites, weed control is critical for the
345 success of valuable broadleaf plantations since it has a decisive effect on survival, growth and vigour of young
346 seedlings. The optimized application of herbicides to late-flushing hybrid walnut gave the best results of all the
347 techniques with regard to tree performance (all 5 years of study) and soil moisture (during dry years). However,
348 mulching proved an effective alternative, especially considering that repeated weeding interventions are
349 obviated, which could be a major advantage in minimal management schemes. The case of biodegradable
350 mulches (biofilm, chips) is particularly beneficial in this regard, as they do not need to be removed at the end of
351 their service life. This advantage, together with their composition based on waste or renewable raw materials,
352 makes them a socially and environmentally valuable alternative to plastic mulching. However, further studies
353 are needed to investigate the optimal properties of biodegradable mulches, both film and particle-based, in
354 various sites, especially in terms of water balance (notably permeability) and durability. We also need to study,
355 from an economic and operational point of view, the relation between the productive outcomes of each
356 treatment and the inputs linked to their repeated application (e.g. herbicide) or need for removal (e.g. plastic
357 mulching), compared with one-time mulch application (e.g. biodegradable models).

358

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Anexo 2. Artículo correspondiente al Ensayo 2.

Use of innovative groundcovers in Mediterranean afforestations: aerial and belowground effects in hybrid walnut

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Abstract - Forest restoration in the Mediterranean area is particularly limited by water scarcity in summer and by weed competition, especially within the first years after establishment. The negative impact of these factors can be mitigated through environmentally friendly and cost-effective techniques which favour root development. This study describes the results of innovative weeding techniques in a reforestation carried out in a former agricultural field in Solsona, NE Spain, under Continental Mediterranean Sub-humid climate conditions. The tested weeding techniques included both novel groundcovers (based on prototypes built on a new biodegradable biopolymer, jute treated with resin and recycled rubber) and reference techniques, i.e. herbicide application, polyethylene and commercial biofilm groundcovers. We studied the response of hybrid walnut (*Juglans x intermedia*) to the application of these techniques during the first vegetative period in terms of survival, aerial growth and aboveground and belowground biomass allocation. The innovative groundcovers produced generally similar outcomes as the reference techniques with regard to tree survival and growth and resulted better in the case of belowground and, to a lesser extent, total tree biomass. Although preliminary, our results suggest that the tested novel groundcovers, notably the model based on treated jute, may represent a promising alternative to plastic mulching and herbicide application in afforestation of agricultural lands under Mediterranean-continental conditions. Besides these promising productive results, the novel groundcovers bring together relevant technical and environmental benefits, related to their use (not requiring removal or being reusable) and composition, based on biodegradable or recycled materials.

Keywords - Biomass allocation, Eco-innovation, Forest restoration, *Juglans*, Mulching, Reforestation

Introduction

Mediterranean climate is characterized by summer drought and high air temperatures (IPCC 2007, Senatore et al. 2011). The annual potential evapotranspiration (PET) often doubles the rainfall, causing significant water stress to plants (Flexas et al. 2006). It is expected that this condition becomes worse in the future because of climate change, as a result of the projected temperature rise and the more intense drought periods (Giorgi and Lionello 2007). Indeed, the Mediterranean region has faced wide climate shifts in the past (Luterbacher et al. 2006), and it has been identified as one of the most prominent 'hotspots' in future climate change scenarios (Giorgi 2006, Ulbrich et al. 2006).

The most critical factors limiting the successful establishment of a new plantation in the Mediterranean context are the high incoming radiation, the air temperature and the low summer rainfall (Verdú and García Fayos 1996). The heavy radiation may lead to photo-damage (Methy 1996) and cause significant soil water losses due to evaporation (Rey Benayas 1998). This factor, together with low summer

rainfall, results in severe water stress particularly critical for young plants with an underdeveloped root system (Coll et al. 2003). Another major factor leading to an enhanced water stress in young trees is the weed vegetation (Rey Benayas et al. 2003). In the Mediterranean area, weeds affect negatively young trees especially with regard to water (Picon-Cochard et al. 2001) and, then, to soil nutrients (Nambiar and Sands 1993) and light availability.

Together with an adequate soil preparation (deep soil ripping, micro-catchments), and the use of adapted seedlings (in terms of species, provenance and root/shoot ratio), there are many techniques that can be applied to facilitate young trees in the first years since the plantation (i.e. the installation phase). These can reduce summer water shortage, exacerbated by weed competition, even though their effectiveness and feasibility depend upon the characteristics of the sites of concern. In the case of competing vegetation, the most common techniques applied in temperate areas are the mechanical and chemical weeding (Willoughby et al. 2009). Mechanical weeding has the disadvantage of requiring a not negligible use of resources and may damage plants

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(George and Brennan 2002). Chemical weeding is an adequate cost-effectiveness solution in many circumstances (Thiffault and Roy 2011). This technique requires however recurrent application and raises significant social/environmental opposition (Bond and Grundy 2001). Its utilization in forest ecosystems is thus increasingly regulated or even banned (Willoughby et al. 2009). Mulching soil with groundcovers is being increasingly considered as a suitable technique alternative to recurrent weeding, especially in the framework of minimizing the number of interventions (Scott Green et al. 2003). Mulching has proven to reduce the vegetative competition in the root zone (Adams 1997) and to mitigate soil water evaporation (McDonald et al. 1994), thus increasing soil water availability. Mulching also increases the availability of nutrients for trees (Wilson and Jefferies 1996). Some mulching models (e.g., films) raise the soil temperature favouring the nutrient cycle and therefore root growth (Ghosh et al. 2006, Kasirajan and Ngouadio 2012).

The application of mulching often results in growth gain in juvenile phase, especially under condition of high vegetative competition (Scott Green et al. 2003). The most common products for film mulching are polyethylene and polypropylene. The main advantage of these materials concerns affordability, easiness of application, long-lasting duration and effectiveness against weeds (Arentoft et al. 2013). Their main drawback is that these products derive from unsustainable raw material (petroleum) and their removal is very expensive (McCraw and Motes 1991). In the last years, bio-based film mulches (i.e. biodegradable and obtained from renewable sources) are becoming available in the market. This allows keeping the advantages of plastic mulches while avoiding the need for removal (Kasirajan & Ngouadio 2012). The main factor limiting the use of biofilm mulches in comparison with plastic-based ones is their higher cost. While a 100 x 100 cm polyethylene sheet can cost 0.4-0.5 €, a similar piece of biofilm may be as expensive as 2-2.5 €.

Goal of the study is to evaluate the effectiveness of new groundcover types in controlling weeds and stimulating seedling growth, both above-ground and below-ground, within the first years in a hybrid walnut plantation in a Mediterranean-continent area. These techniques were developed with the aim of improving forest restoration projects in Mediterranean and temperate conditions from an environmental, technical and economic viewpoint. Our hypothesis is that the new groundcovers should increase tree growth compared to the unweeded trees, similarly to the reference weeding techniques (i.e. plastic mulching and herbicide application).

Materials and methods

Study area and plantation description

The study was carried out in Solsona, Lleida, NE Spain, at coordinates 42°00'09.71"N 1°31'46.09"E, elevation of 672 m a.s.l.. The climate is Mediterranean-continent sub-humid (Martín-Vide 1992), with an average annual temperature of 12.0°C (average temperature at the warmest and coldest month is 21.4°C and 3.7°C, respectively). Mean annual precipitation is 683 mm, 165 mm during summer period (Ninyerola et al. 2005). The plantation was established in spring 2014 in a flat field, previously used for cereal production (wheat and barley).

Soil texture is loamy-clay (32% clay, 40% silt and 28% sand). During the plantation the soil was prepared by crossed sub-soiling with ripper (45 cm depth). Plants were installed manually, in pits sized 40 x 40 x 40 cm. The plantation scheme was regular, 3 x 3 m. The vegetative material was hybrid walnut (*Juglans x intermedia*) MJ-209xRa, bare rooted, 40/60 cm high. Aim of the plantation was the production of valuable timber for veneer industry.

Experimental design and treatments

The seven experimental treatments, described in Table 1, include three innovative groundcovers, three reference techniques against competing vegetation and a control (unweeded trees). These treatments were compared following a randomized block design with 6 blocks, each of them containing 10 trees per treatment (60 trees per treatment, 420 experimental trees).

Table 1 - Description of the 7 experimental treatments. Each individual treatment refers to 80x80 cm area.

| Treatment type | Description | Treatment code |
|------------------------------|-------------------------------------------------------------------------------------------------------------------|----------------|
| Control | *No weeding treatment | Unweeded |
| Reference weeding techniques | * Herbicide application (glyphosate, 14.4 cm3 tree-1 at 1.25%) applied in May via backpack sprayer | Comm_HER |
| | *Commercial black polyethylene film, anti-UV treated, 80 µ thick | Comm_PE |
| | * Commercial green biodegradable woven biofilm | Comm_BF |
| Innovative groundcovers | * Recycled rubber based mulch, anti-UV treated, long-lasting and not requiring fixation (1.5 mm thick) | New_RUB |
| | * Woven jute cloth treated with bio-based resin for increased lifetime, 100% biodegradable | New_JUTE |
| | * Black new biopolymer-based frame 100% biodegradable, fused to a black commercially available biodegradable film | New_BF |
| | | |

Data collection and measurements

Survival and vegetative status

Tree mortality and vegetative status were as-

sessed visually in October 2014. A tree was considered to reveal vegetative problems when the apical shoot was dead or when showing basal sprouting.

Stem growth

Basal diameter (measured with digital caliper 5 cm over the ground level on a painted mark) and total height (by measuring tape) were collected both at planting (April) and at the end of the vegetative period (October). Annual diameter and height increment were calculated as the difference between first and second measurement.

Biomass allocation study

Two living trees per treatment and block (84 in total) were randomly chosen in November 2014. These trees were pulled out carefully by a small bobcat-type backhoe excavator, keeping a full rootball with all fine roots intact. Uprooted trees were immediately placed in labelled paper bags and stored at 4°C until being processed at the lab within the subsequent 7 days. Following Schall et al. (2012) and Nanayakkara et al. (2013), the roots were put in a bucket with water to soften them and dilute soil and then rinsed gently with tap water, without damaging the roots and recovering all broken roots from each tree. Then, the trees were divided into three components: stem, coarse roots and fine roots (thickness >2 and <2mm, respectively), by using scissors for cutting and digital caliper for measuring thickness. Each component was located into a labelled aluminium tray, where they were oven-dried at 70°C for 72 hours. Finally, dry components were weighed. The variables resulting from this process are the absolute (g) and relative (%) with respect to the total of the tree) biomass for each component.

Weather in 2014

The study took place within the 2014 growing season, characterized by an anomalous high rainfall amount in the July-September months (313 mm), value that almost doubled the average historical reference value of 165 mm. There was no relevant dry period during the year according to the Bagnous & Gaussen diagram from January to October 2014 (Fig. 1).

Data analysis

Data analysis consisted of evaluating the effect of the different weeding techniques. The survival and vegetative status were analyzed by means of the Pearson's Chi-square (χ^2) test. Tree growth and biomass allocation were analyzed through a one-way analysis of variance (ANOVA; p-level < 0.05). When significant differences between treatments resulted from ANOVA, these were evaluated by

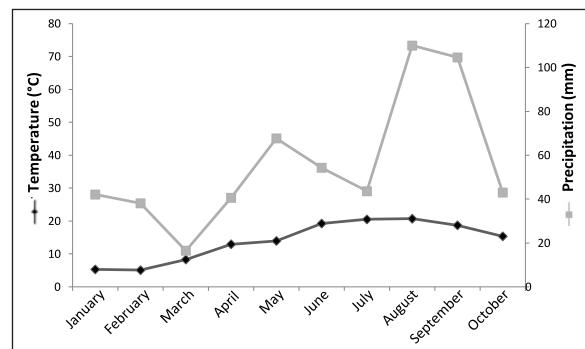


Figure 1 - Bagnous & Gaussen diagram for the vegetative period 2014 in the study area.

means of post-hoc Tukey's HSD test. The analysis was performed with the software Statistica 7.1, 2005 (StatSoft Inc. USA).

Results

Survival and vegetative status

During the first vegetative period overall mortality rate was very low (five trees or 1.2%) with the higher mortality rate in the unweeded trees (four trees out of five). As for the vegetative status, 16.7% of trees showed some kind of vegetative problem (Fig. 2). The Pearson's χ^2 test did not explain any relationship between treatment and incidence of mortality or vegetative problems.

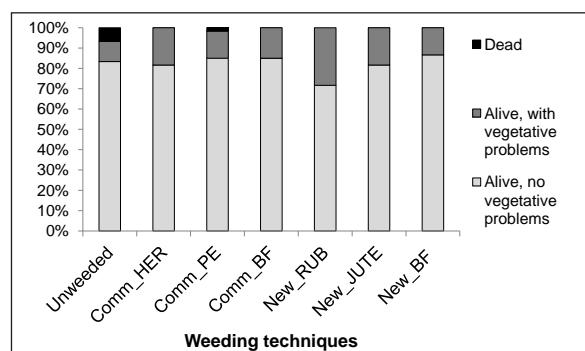


Figure 2 - Percentage of trees under each category of survival and vegetative status.

Aerial tree growth: diameter and height

Aerial growth (both in diameter and height) was affected by the different weeding treatments applied. Diameter growth was significantly improved by all the weeding treatments compared to the Unweeded-control, with diameter values which were doubled or tripled. However, no significant difference was found between the 6 weeding techniques tested (Fig. 3).

Height growth showed the same trend, with Unweeded-control providing the lowest values, although only New_JUTE and New_BF provided significantly higher results (Fig. 4).

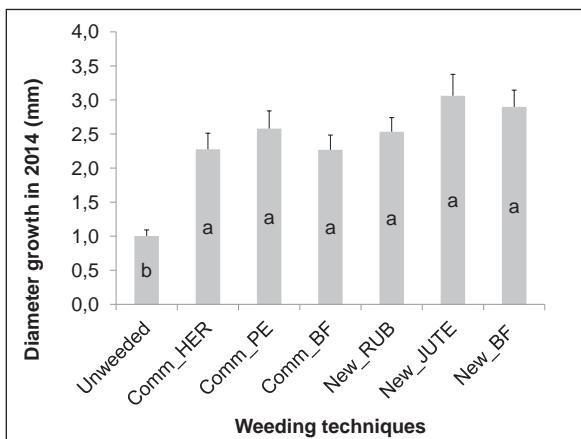


Figure 3 - Mean walnut diameter growth in 2014, subject to different weeding techniques; whiskers indicate standard error of the mean. Different letters indicate significant differences at the $p<0.05$ level, grouping according to Tukey's HSD post hoc test.

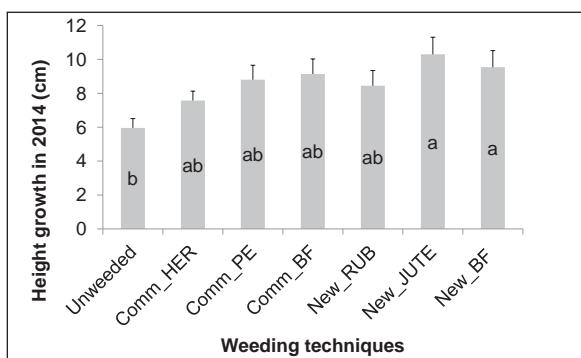


Figure 4 - Mean walnut height growth in 2014, subject to different weeding techniques; whiskers indicate standard error of the mean. Different letters indicate significant differences at the $p<0.05$ level, grouping according to Tukey's HSD post hoc test.

Biomass allocation

The weeding treatments affected the allocation of biomass among the different tree components, both for absolute (total biomass per component) and relative (percentage of biomass for each component with respect to total tree biomass and root/shoot ratio) variables considered. All innovative weeding techniques (New_RUB, New_JUTE, New_BF) led to significantly higher biomass than Unweeded-control for all tree components, while Comm_BF improved biomass production in coarse roots, total roots and total biomass in comparison to Unweeded-control (Figure 5). The comparison between the different weeding treatments highlights a consistent trend: New_JUTE and New_BF resulted in higher biomass allocation than all three reference techniques for most compartments, while New_RUB only improved biomass production with respect to Comm_PE and Comm_HER at most cases.

In the case of the relative distribution of biomass among the various compartments, Unweeded and Comm_PE provided a lower share of biomass in coarse roots than most of other treatments, and a higher proportion of fine roots and aboveground biomass (Fig.6).

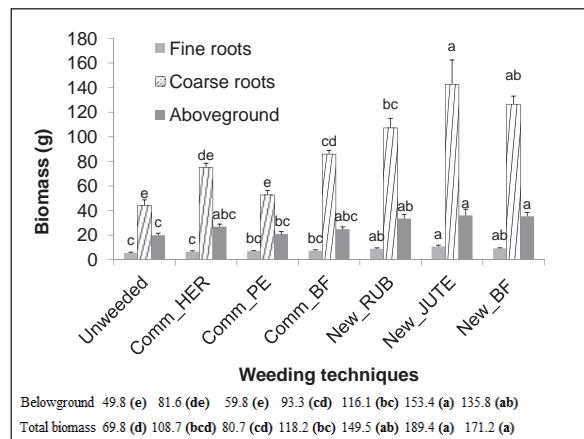


Figure 5 - Mean biomass allocated to each tree compartment (g) as a response to the different treatments. Whiskers indicate standard error of the mean. Different letters indicate significant differences at the $p<0.05$ level, grouped according to Tukey's HSD test. Below the graph are given the mean total root biomass (belowground) and total seedling biomass, with the letters in brackets showing the grouping according to Tukey's HSD test.

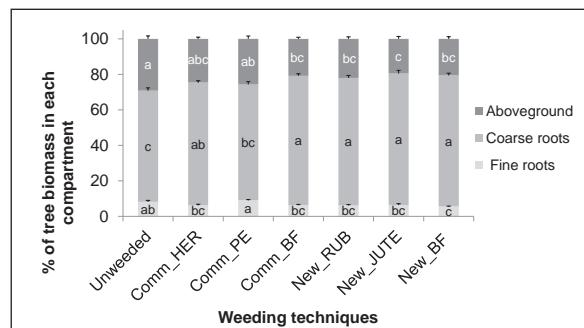


Figure 6 - Mean relative allocation of biomass in each compartment, with respect to total tree biomass (%); whiskers indicate standard error of the mean. Different letters indicate significant differences ($p<0.05$), grouped by Tukey's HSD test.

Weeding techniques had a significant effect on root/shoot ratio. Also for this parameter New_JUTE showed significant higher values than Comm_PE, while both New_JUTE, New_BF and Comm_BF provided significant higher values than Unweeded (Fig.7).

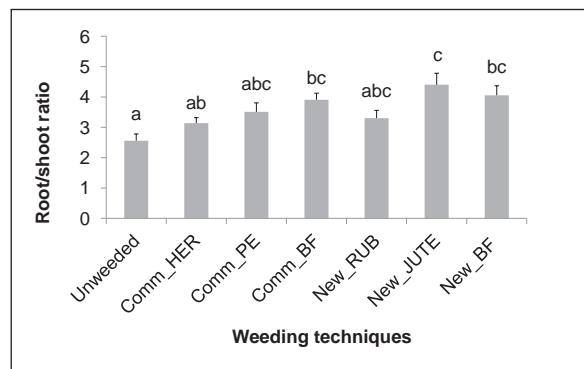


Figure 7 - Mean root/shoot ratio for the different weeding treatments; whiskers indicate mean standard error. Different letters indicate significant differences ($p<0.05$), grouped by Tukey's HSD test.

Discussion

The analysis highlighted the effect of different

weeding techniques on survival, vegetative status, aerial growth and biomass allocation of hybrid walnut seedlings, during the first growing season. We found a positive effect of all weeding treatments in most variables, compared to the unweeded trees.

Seedling survival and vegetative status

The survival of seedlings after one growing season is high (98.8%). Among the five dead plants of the study, four corresponded to the Unweeded treatment, suggesting the negative effect of competition (Chaar et al. 2008). The vegetative status was generally good, 82.1% of seedlings being healthy. No linkage was checked between the different treatments applied and the occurrence of vegetative problems.

Tree aerial growth

Weeding techniques had a remarkable positive effect compared to unweeded trees (Haywood 2000, Athy et al. 2006). Diameter growth of all weeding treatments resulted to be superior to unweeded trees as already observed in other research trials (Bendfeldt et al. 2001, Paris et al. 2005). Tree height growth, in New_JUTE and New_BF showed higher values than unweeded trees, as observed by previous studies on walnut (Haywood 1999, Smith et al. 2000). Significant tree growth differences between weeded and unweeded trees but not significant differences among different weeding techniques was also found in other analyses (Abouziena et al. 2008, Maggard et al. 2012).

Absolute biomass allocation

The analysis of biomass allocation among the different tree compartments (fine roots, coarse roots, aerial biomass) showed a clear effect of the different treatments applied. All innovative mulching techniques, and Comm_BF in most cases, increased the biomass of all tree compartments compared to unweeded trees, as found in previous studies assessing mulching techniques (Scott Green et al. 2003). By excluding weed competition, mulching increases water, nutrients and light available for the trees (Wilson and Jefferies 1996, Liu et al. 2003), and in turn facilitates an increase in biomass productivity (Haywood 1999). The case of root biomass is especially relevant in the study of young tree plantations, since it allows the exploration of larger soil volume and increases its resistance to prolonged water stresses in the subsequent years (Vallejo et al. 2012). The innovative mulches improved seedling rooting during the installation phase compared to Unweeded and reference weeding techniques.

Only one mulching technique (Comm_PE) did not increase significantly tree biomass of any compartment compared to unweeded trees. The

reason could be related to the particular features of polyethylene mulch, i.e. a continuous plastic layer fixed to the ground, thus limiting water and air circulation and considerably reducing the soil water evaporation (Sharma et al. 1998). In general, this feature is an advantage when water availability is the main limitation (McCraw and Motes 1991) linked to drought and/or poor soil water retention. However, in the case of concern, the trial took place on a heavy textured soil and under a very wet year, and the same feature may have resulted in excessive water accumulation in the soil, which is in turn an undesirable condition for walnut (Becquey 1997). Indeed, the best results were obtained by New_JUTE mulch, a woven-structure mat with rather high permeability to water, allowing infiltration during a rainfall event and evaporation afterwards thus enhancing tree growth (Debnath 2014). The effectiveness of jute mulching has also been demonstrated in other studies, especially in agriculture (Tomar et al. 2005) not only for its weed control potentialities, but also for its mechanical and hydraulic characteristics, even concerning the regulation of soil temperature (Sanjoy 2014).

Comm_BF, leading to slightly better results than the other reference techniques, is also a woven mat with a similar structure as New_JUTE (55 threads cm⁻² in front of 58 threads cm⁻², respectively) but with a notably different density (110 g m⁻² in front of 460 g m⁻²) and hygroscopic properties: while Comm_BF does not absorb water, New_JUTE consists of vegetal fibres which need to be moistened before becoming permeable. As a result, Comm_BF may allow a slight rainfall to penetrate in the soil but its capacity to prevent its evaporation will not stand for long time. On the other hand, New_JUTE is expected to require a heavier rainfall to allow water to reach the soil, but it would be therefore retained for longer time. In our study, with a rather high supply of water, New_JUTE performed better than Comm_BF in terms of belowground biomass, probably linked to a more efficient prevention of soil water evaporation during the warmest periods of summer, while it still allows sufficient soil aeration, opposed to Comm_PE.

Regarding New_BF and New_RUB, they are both black film mulches, although contrary to Comm_PE their surface is not continuous but consists on assembled pieces which leave some air exchange through the film, although closed enough as to impede weed growth through the mulch.

Finally, Comm_HER did not improve the results of unweeded trees regarding biomass production of any tree component. In contrast, the use of the same herbicide with the same concentration and time of application yielded during four years (2011-2014)

created much more favourable results than the use of four different mulching models in a trial located two kilometers away from the field of this study, with similar soil features and the same tree species (Coello et al. *submitted*). In the mentioned study, the area per tree treated with herbicide was 100 x 100 cm, while in our study the treated area was similar to mulch size (80 x 80 cm), thus one third smaller. Moreover, in our study the wet conditions in 2014 might have boosted weed proliferation and its competitiveness during summer, reducing the efficiency of a one-time, spring herbicide application.

Relative biomass allocation

The survival of seedlings during summer is closely related to the development of the root system, rather than to the aerial organs (Lloret et al. 1999, Villar-Salvador et al. 2012). A more developed root system can more easily absorb deep (Canadell and Zedler 1995, Pemán et al. 2006) and surface water (Hilbert and Canadell 1995), and allows a more efficient extraction of nutrients (Wein et al. 1993, Lambert et al. 1994). The root/shoot ratio is a trait describing how the tree distributes the available resources (Lamhamadi et al. 1998), it being very important under water-limited condition, like Mediterranean climates (Lloret et al. 1999). It is generally accepted that a high root/shoot ratio indicates a better chance of survival under Mediterranean conditions (Navarro et al. 2006, Jacobs et al. 2009). Plants with a high root/shoot ratio are considered as better performers in water-limited sites (Royo et al. 1997), as they consume less water than plants with opposite traits (Leiva and Fernández-Ales 1998). In our study, the root/shoot ratio was found to be especially high for the treatments resulting in highest biomass production of compartments altogether (New_JUTE and, to a lesser extent, New_BF and Comm_BF).

Fine roots and coarse roots are the components of belowground biomass, their dry weight being proportional to the volume of soil that they can explore (Tufekcioglu et al. 1999). Fine roots represent a dynamic portion of belowground biomass, i.e. the main component dedicated to nutrients uptake, and representing a significant part of net primary production (Buyanovsky et al. 1987). On the other hand, coarse roots production is closely linked to resource availability (Albaugh et al. 1998) and especially involved in carbohydrate and nutrient storage (Comas et al. 2013) making the tree more resistant to stresses. In our study, mulching technology, particularly the new versions, enhanced a higher production of root biomass, especially among the coarse component, thus being an indicator of successful plantation.

Conclusions

Our preliminary study demonstrated the importance of weeding for increasing both aerial and belowground early tree growth in productive Mediterranean-continental conditions.

Mulching was especially effective on reducing competition by herbaceous species for water and nutrients. Among the mulching techniques tested, a novel model based on woven jute proved to be particularly successful, with enhanced results for all the traits studied, probably associated with its adequate permeability rate, which was beneficial in a wet year as 2014. Other innovative film mulches based on new biopolymers and on recycled rubber, and to a lesser extent a commercial biofilm, also led to superior results in comparison to unweeded trees, and to trees subjected to herbicide application or to polyethylene mulching.

The evaluated novel mulches demonstrated their technical adequacy, in addition to the environmental advantages compared to reference techniques; both the new biopolymer formulation and the treated jute films are 100% biodegradable, this avoiding the need for their removal and being a notable advantage compared to plastic groundcovers. The new mulch based on recycled rubber is not biodegradable, but it is made of recycled waste and its long durability (estimated in up to 15 years in outdoor conditions) makes it especially suitable for long-term applications where removal costs should be minimized: urban forestry, afforestation of easily accessible land, etc.

These results correspond to a preliminary phase of study, and must be confirmed with data from further years and further study areas in order to adequately assess the potential of these techniques for forest restoration under Mediterranean conditions with regard to their effect on tree survival and growth.

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Anexo 3. Artículo correspondiente al Ensayo 3.

1 **Title:** Innovative soil conditioners and mulches for forest restoration in semiarid
2 conditions in northeast Spain

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7 **Abstract**

8 Restoring degraded ecosystems is a global challenge. Wherever applicable, forest restoration is
9 one of the most effective tools for reversing degradation processes and enhancing multiple
10 ecosystem services. In Mediterranean semiarid conditions the main limiting factor for tree
11 establishment is the low and irregular precipitation regime, which has a particularly harmful
12 effect in areas where the soil has a poor water retention capacity. We tested, alone and
13 combined, two types of cost-effective and locally applied plantation techniques that aim to
14 promote early seedling establishment: i) various mulches including biodegradable and reusable
15 prototypes and commercial models; ii) two soil conditioners with water-superabsorbing
16 polymers in their formulation, one of which includes a new polyacrylamide-free polymer, which
17 was tested at various doses. In a three-year study we examined their effects on *Pinus halepensis*
18 performance (survival, shoot and root growth and tree water status) and on soil moisture on a
19 north-facing and a south-facing slope in Mequinenza, NE Spain. The use of mulches led to
20 slight increases in seedling growth and soil moisture compared to untreated seedlings, without
21 great differences between the models tested. Therefore the new prototypes can be considered as
22 suitable alternatives to commercially available ones. On the other hand, the new soil conditioner
23 led to much clearer positive effects. Compared to untreated seedlings, the new soil conditioner
24 improved seedling survival, root and shoot growth and water status, as well as soil moisture.
25 The benefits of the new soil conditioner were highest when applied at doses of 40 or 80 g per
26 seedling. We found that this new formulation achieved similar performance as the commercially
27 available one. Combining mulches and soil conditioners resulted in additive outcomes, rather
28 than in synergistic ones. We conclude that in conditions limited by low precipitation and coarse
29 textured soils the use of small mulches does not seem a priority technique, in contrast with the
30 application of soil conditioners, which seems an effective option for enhancing early seedling
31 performance.

32 **Keywords:** afforestation; Mediterranean; *Pinus halepensis*; reforestation; water-absorbing
33 polymer

34 **Abbreviations:** RWC = Relative water content; SAP = Super-absorbing polymers; SCwSAP =
35 Soil conditioner with super-absorbing polymers

36 **1. Introduction**

37 Land degradation affects more than 2 billion hectares worldwide (Potapov et al. 2011), with a
38 range of drivers varying among regions. In the Mediterranean basin land has been overexploited
39 for millennia (Blondel and Aronson 1999), which has involved massive land use changes for
40 promoting agriculture and grazing in areas recurrently affected by wildfires (Shakesby, 2011).
41 This has put many areas under threat of erosion and desertification. In these conditions, and
42 particularly in the semiarid areas, the spontaneous recovery of the forest cover is limited by the
43 slow growth dynamics linked to irregular and low water availability and high evapotranspiration
44 rates (Vallejo et al. 2012). These conditions are expected to worsen in the coming decades due
45 to the forecasted rise in temperatures and heat waves and the decrease in precipitation in this
46 area (IPCC, 2014). The spontaneous recovery of these areas is severely limited due to the
47 cumulative effects of drought, wildfires and soil erosion and will strongly depend on weather
48 and site conditions such as soil features, slope steepness and aspect (Alrababah et al. 2007).

49 At present, there is a wide range of eco-technological tools used to restore semiarid
50 environments that make it possible to improve (micro)site conditions, resource availability and
51 the capacity of plants to endure stress (Cortina et al. 2011), particularly during their first years
52 (Vallejo et al. 2012).

53 One of these tools are soil conditioners, i.e. products mixed with the soil in the planting pit to
54 improve the soil chemical and/or physical properties at micro-site level for improving early
55 seedling performance (Coello and Piqué, 2016). The application dosage has a major effect on
56 the cost and the performance of this technique (Del Campo et al. 2011) and therefore it should
57 be tuned up to balance its cost-effectiveness. One of the most successful components of soil
58 conditioners are water superabsorbent polymers (SAP), also referred to as hydrogels or
59 superabsorbers, synthetic compounds that can absorb up to 400 times their weight in water
60 (Rowe et al. 2005). The use of SAP – alone or combined with fertilizers and other components –
61 has proven effective in agriculture and forestry, increasing soil water availability, reducing
62 evaporation and enhancing early survival and growth in a wide range of species (Hüttermann et
63 al. 2009). Most SAP are based mainly on cross-linked polyacrylamide, which is becoming less
64 socially accepted because of the potential traces of unpolymerized acrylamide. Despite being
65 considered environmentally compatible (Holliman et al. 2005; Hüttermann et al. 2009) and
66 meeting the legal limits of free acrylamide, producers are developing new, polyacrylamide-free
67 SAP (DRI, 2008); however, their optimal dosage and effectiveness in the field is yet to be
68 established.

69 One limitation of soil conditioners and similar techniques in afforestation is that the
70 improvement in site conditions often enhances competition from spontaneous vegetation,
71 masking the potential benefits of this technique and increasing the need for weeding (Cogliastro
72 et al. 2001, Fuentes et al. 2010). A possible solution is the use of mulches, also known as
73 groundcovers or weeding mats, to reduce competition from unwanted vegetation. This
74 technique involves covering the soil around the seedlings with an opaque layer that impedes
75 weeds from germinating and becoming established near the seedling (Maggard et al. 2012). In
76 addition to weed control, mulches regulate soil temperature and reduce soil water evaporative
77 losses, thus increasing soil moisture (Benigno et al. 2013, Jiménez et al. 2014). They also
78 improve soil aggregate stability and nutrient availability (Jiménez et al. 2016), which ultimately
79 limits soil erosion. These factors have increased the interest in this single-application technique
80 as an alternative to recurrent chemical or mechanical weeding (Coello et al. 2017). The wide
81 range of mulch materials, colors and structures available allows fine-tuning the desired
82 properties with regard to water and air permeability and temperature dynamics.

83 The most common mulching material is plastic, because of its low retail, transport and install
84 costs (Arentoft et al. 2013). However, it has as main drawbacks its unsustainable origin, poor
85 aesthetic value and the need to be removed at the end of its service life to avoid polluting soil
86 and water. To tackle these limitations there is an incipient availability of biodegradable mulches
87 in the market, made of renewable raw materials i.e. vegetal fibers and bio-based plastics
88 (Álvarez-Chávez et al. 2012) and that do not result in a negative impact during their
89 degradation. Another approach to enhance the sustainability of mulching is the use of waste or
90 recycled products as raw materials, in the framework of a circular economy (European
91 Commission, 2015). Many of these new options are at the prototype stage and require field
92 testing to assess their potential.

93 The combined application of mulches and soil conditioners would make it possible to address
94 the five priority factors proposed by Cortina et al. (2011) for field techniques that aim to
95 improve seedling establishment: increase (i) the rootable soil volume, (ii) nutrient availability,
96 (iii) runoff collection, (iv) water conservation and (v) control competition from extant
97 vegetation. Although the combined application of mulches and soil conditioners with SAP
98 (SCwSAP) seems promising, only few studies have analyzed the joint effect of these techniques
99 on seedling performance and soil parameters, on broadleaved species (Navarro et al. 2005).
100 Furthermore, SCwSAP containing polyacrylamide-free SAP have not yet been field-tested.

101 In this study we tested different combinations of five mulches, three of which were at the
102 prototype stage, and various SCwSAP applications: a commercial one, containing

103 polyacrylamide, and a new polyacrylamide-free formulation at various doses. We assessed their
104 effectiveness in promoting early seedling performance and soil moisture in conditions limited
105 by water shortage as a result of a semiarid climate and a poor, coarse-textured soil at two sites: a
106 N-facing and a S-facing slope. We tested their effect on Aleppo pine (*Pinus halepensis*)
107 seedlings, the most abundant species in semiarid conditions in the western Mediterranean
108 (Quézel, 2000) in terms of distribution and use for afforestation purposes (Rincón et al. 2007).
109 We hypothesized that:

- 110 i) both mulches and SCwSAP should have a positive effect on seedling performance and soil
111 moisture, while the combined use of both techniques should lead to a synergistic performance;
112 ii) the performance of SCwSAP should be proportional to the application dose, which should
113 allow determining the most cost-effective dosage;
114 iii) the commercial and the new SCwSAP should have a similar performance when applied at
115 the same dose.

116 **2. Materials & methods**

117 **2.1. Study area and weather summary**

118 The study area is located in Mequinenza (Aragón region, inland northeast Spain, 41.3374N;
119 0.1429E) and has a semiarid climate (mean annual temperature = 15.0 °C, annual rainfall = 367
120 mm, Köppen classification: BS – Steppe cold). The mean altitude is 198 m o.s.l. We installed a
121 twin trial in two nearby sites (500 m from each other): the first S-facing (aspect 210°) and the
122 second N-facing (aspect: 30°), with a total area of 1.2 ha. Both plots are on steep slopes (40%
123 and 50% inclination respectively). The soil is a Calcisol (FAO, 2015), with a sandy-loam
124 texture, pH 7.9 and scattered gypsum veins.

125 During the study period, temperatures were warmer than the historical reference (Ninyerola et
126 al. 2005). The annual precipitation was mostly in line with the reference values, although the
127 summer precipitation varied drastically from year to year (Table 1). Daily temperature and
128 precipitation data were obtained from the nearest weather station of the Catalan Meteorological
129 Service, representative of the study site.

130 Table 1. Summary of reference (Ninyerola et al. 2005) and annual values of the main
 131 meteorological features at the study site. GS_n indicates the correlative growing season.

| Year | Mean daily temperature in summer (°C) | Mean maximum daily temperature in summer (°C) | Annual precipitation (mm) | Summer precipitation (mm) | Summer precipitation events >10 mm (#) |
|-----------------------|---------------------------------------|-----------------------------------------------|---------------------------|---------------------------|----------------------------------------|
| Reference (1951-1999) | 23.7 | 30.6 | 367 | 69 | - |
| 2014 (GS1) | 24.2 | 31.8 | 370 | 62 | 2 |
| 2015 (GS2) | 25.8 | 33.1 | 330 | 120 | 3 |
| 2016 (GS3) | 25.0 | 31.9 | 361 | 11 | 0 |

132

133 The whole area was covered by Aleppo pine (*Pinus halepensis*) and had been affected by a high
 134 intensity wildfire in summer 2003. At the beginning of the experiment (2014) the area showed
 135 very poor spontaneous recovery, limited to scattered bushes of *Quercus coccifera*, *Pistacia*
 136 *lentiscus* and *Rosmarinus officinalis*. In the least covered areas, which were predominant in the
 137 S-facing trial, there were erosion problems including active ravines and gullies.

138

139 2.2. Experimental design

140 We applied the same design in the two trials: a randomized incomplete block design. Each trial
 141 consisted of six blocks, each including 75 seedlings that were randomly assigned to one of the
 142 15 possible treatments (5 replicates per treatment per block). Treatments consisted of
 143 combinations of various mulch models and soil conditioners. In total we planted 450 seedlings
 144 (30 per treatment) in each trial. Table 2 shows the description of each technique (mulch and soil
 145 conditioner) applied.

146 Table 2. Description of the experimental techniques.

| Technique | Technique | Description |
|---------------------|------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| type | code | |
| Mulch | Control | No mulch applied |
| | Com_Plastic | Commercial black polyethylene film, anti-UV treated, 80 µm thick. |
| | Com_Biofilm | Ökolys®, a commercial green biodegradable woven mat |
| | New_Biofilm | Prototype of black biodegradable frame (biopolymer), fused to a black commercial biodegradable film based on PHA (polyhydroxyalkanoate), 80 µm thick, manufactured by Groencreatie and DTC. The frame is to make installation easier. |
| | New_Jute | Prototype of biodegradable woven jute mat treated with furan bio-based resin for increased durability, manufactured by La Zeloise NV. |
| | New_Rubber | Prototype of black layer made of recycled rubber, anti-UV treated, 1.5 mm thick to make fixation unnecessary, manufactured by EcoRub BVBA. |
| Soil conditioner | SC- | No soil conditioner applied |
| | New_SC20; | TerraCottem Arbor®, at the prototype stage when tested. Product developed for |
| | New_SC40; | tree and shrub planting. Its formulation includes a new generation of |
| | New_SC80 | polyacrylamide-free water absorbent polymers (36.25% of total weight), volcanic rock (48.25%), fertilizers (14.5%; NPK 3-1-7), humic acids (0.75%) and growth precursors (0.25%). 20, 40 and 80 indicate the dose applied (g seedling ⁻¹) |
| | Com_SC40 | TerraCottem Universal®, a commercially available soil conditioner with cross-linked polyacrylamide and polyacrylic acid, fertilizers and volcanic rock. The dosage was 40 g seedling ⁻¹ |

147

148 The 15 treatments were organized in three sub-experiments:

149 (i) Sub-experiment 1: a full factorial design combining the 6 different mulch treatments with
150 New_SC applied at a dose of 40 g seedling⁻¹ (New_SC40) or with a control (SC-). Overall there
151 are 12 treatments with 30 seedlings per treatment, thus 360 seedlings in total. The soil
152 conditioner dose of 40 g seedling⁻¹ corresponds to the manufacturer's recommendation for the
153 most similar commercial product available.

154 (ii) Sub-experiment 2: a study on the effect of four different doses of New_SC (0, 20, 40 and 80
155 g seedling⁻¹), combined with a reference mulch (Com_Plastic) in all cases. Each of these four
156 treatments comprises 30 seedlings, for a total of 120 seedlings in this sub-experiment.

157 (iii) Sub-experiment 3: a study comparing a commercial and a new SCwSAP (Com_SC vs.
158 New_SC), both applied at a dosage of 40 g seedling⁻¹ and combined with a reference mulch
159 (Com_Plastic). Each of the two treatments comprises 30 seedlings, for a total of 60 seedlings.

160 2.3. Seedling planting

161 In each field trial we planted 450 seedlings of Aleppo pine in early March 2014. We performed
162 mechanical soil digging with a spider backhoe excavator in order to minimize the impact on the
163 soil. The volume of soil stirred (not removed) was 40 x 40 x 40 cm, shaped as micro-basins to
164 collect runoff and avoid erosion. As the land was uneven we deployed the plantation pits in
165 random locations, with at least 3 m between two consecutive pits. We used one-year-old *Pinus*
166 *halepensis* seedlings, 15-25 cm high in containers of 300 cm³ as proposed by Puertolas et al.
167 (2012), from the Spanish Provenance Region 03 (*Inner Catalonia*), which fitted the local
168 conditions. The seedlings showed an overall good health status at the moment of planting. We
169 applied the SCwSAP manually during planting, in sub-pits sized 30 x 30 x 30 cm, following the
170 manufacturer's instructions: half of the dose was applied at the sub-pit bottom and the
171 remaining half was mixed with the earth utilized to fill up the sub-pit. After planting, we
172 installed the mulches, which were chosen with small dimensions (40 x 40 cm), aiming to adapt
173 the costs (retail, transport, installation) to the expected poor weed competitiveness. The mulch
174 dimensions were also similar to the area of planting pits (40 x 40 cm).

175 2.4. Seedling survival and growth monitoring

176 We monitored all seedlings to determine their survivorship (visual assessment: alive or dead),
177 basal diameter and total height annually at the end of the first three growing seasons (Octobers
178 2014-2016; GS1-GS3, hereinafter). We measured basal diameter at a constant point marked on
179 each seedling when they were planted. We conducted additional survival monitoring six weeks
180 after planting to detect short-term dead seedlings whose failure could not be attributed to the
181 treatment but rather to poor plant quality or an inappropriate planting operation. We removed
182 these seedlings (21 in total) from the experiment.

183 2.5. Biomass allocation

184 At the end of the first growing season we pulled up one live seedling per treatment and block (n
185 = 6; 90 seedlings per field trial), chosen randomly, with the root system intact, in order to study
186 biomass allocation. We washed the root system and then divided the seedling into three
187 components: fine roots (< 2 mm diameter), coarse roots (> 2 mm) and shoot (stem and needles).
188 We dried the samples at 60°C for 72 h to obtain the dry mass of each component. Because we
189 obtained similar results for fine and coarse roots in all tests, we decided to aggregate them into a
190 single variable, root mass.

191 2.6. Seedling water status

192 We measured the needle relative water content (RWC, hereinafter) six times: July GS1 (2
193 measurements), August GS1, September GS1, July GS2 and August GS2. In each measurement
194 we collected, from each trial, treatment and block ($n = 6$), one composite sample consisting of
195 15-20 needles from at least two different seedlings. Therefore in each measurement we collected
196 90 samples per trial, which were placed in hermetic plastic vials stored in a portable cooler
197 immediately after collection. On the same day we measured the fresh mass of needles (FM) in
198 the laboratory, and put them in distilled water for 18 h for full hydration. We then measured the
199 saturated mass (SM). Finally, we dried the needles at 60°C for 48 h to obtain their dry mass
200 (DM). We calculated the needle relative water content (RWC) as: % RWC = 100 * (FM - DM)
201 * (SM - DM)⁻¹.

202

203 2.7. Soil moisture monitoring

204 We measured soil moisture from May to September during GS1 and GS2 (6 and 5
205 measurements respectively) and monthly during summer GS3, for a total of 13 measurements.
206 Six of these dates coincided with the seedling water status measurements. We took the
207 measurements at three constant points per treatment ($n = 3$; 45 sampling points per field trial) at
208 two depths (0-20 and 20-40 cm). Sampling points were located 7.5-10 cm away from the
209 seedling, and consisted on access tubes installed right after planting, through which a TDR
210 probe (Trime-Pico T3, IMKO) was guided.

211 2.8. Statistical analyses

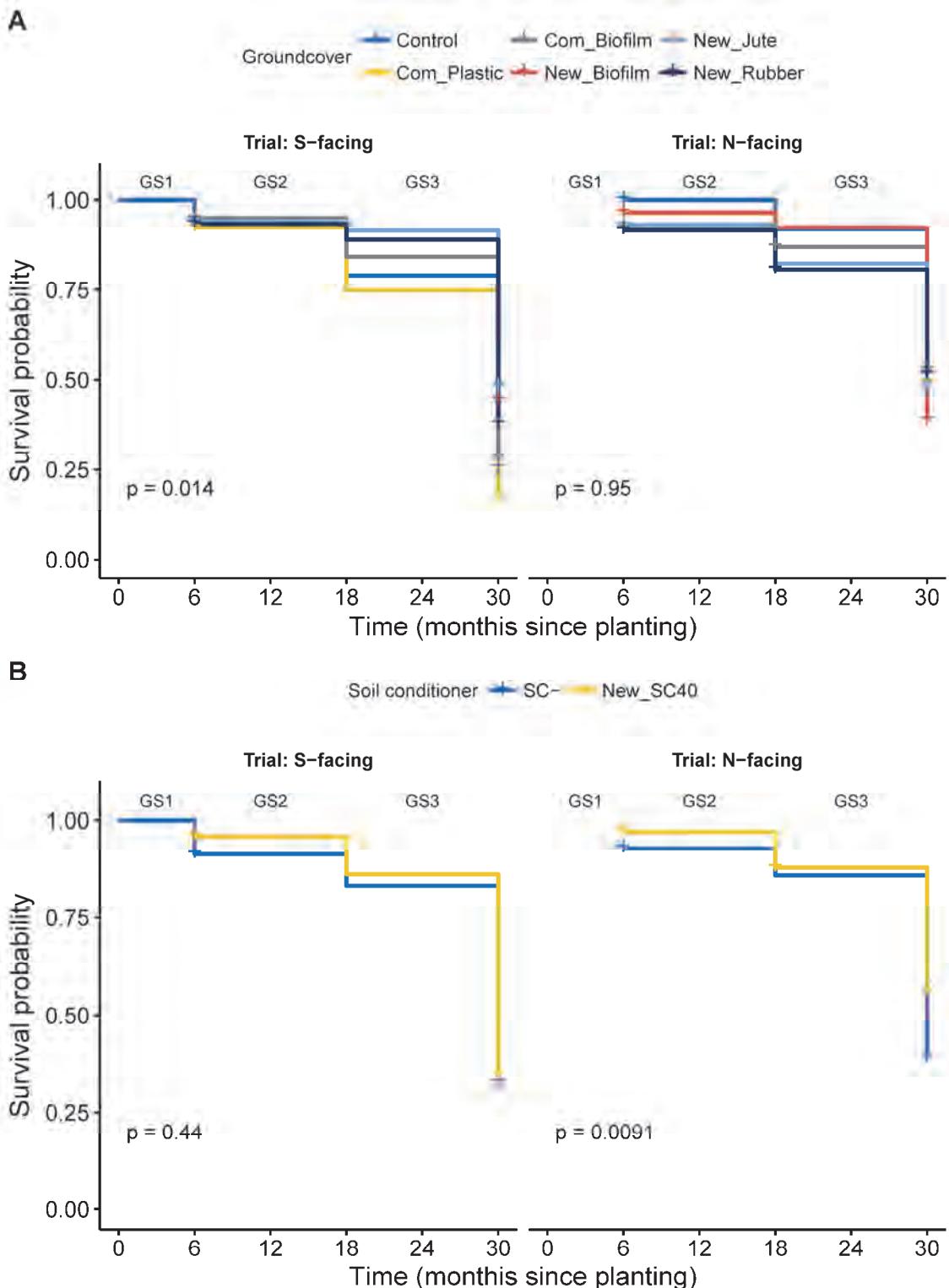
212 We analyzed the data independently for each trial and sub-experiment, considering treatment as
213 fixed factor and block as random factor. In the case of RWC and soil moisture at each depth we
214 considered the data from all the measuring dates (6 and 13, respectively) altogether in order to
215 have a robust dataset.

216 For survival analyses, we built survival curves for the three first growing seasons based on
217 Kaplan-Meier estimates (Kaplan and Meier, 1958), and we used the Mantel-Cox log-rank test to
218 determine significant differences between treatments. We used ANOVA to assess the
219 differences between treatments in seedling annual diameter growth and height growth ($n = 30$ in
220 GS1; $n = 24$ in GS2-3), biomass allocation ($n = 6$), RWC ($n = 6$) and soil moisture ($n = 3$ for
221 each depth). We used two-way ANOVA for sub-experiment 1 (mulch, soil conditioner,
222 interaction mulch x soil conditioner) and one-way ANOVA for sub-experiments 2 (soil
223 conditioner dosage) and 3 (soil conditioner formulation). We assessed pairwise differences
224 between treatments with the post-hoc Duncan's multiple range test. Height growth, biomass
225 allocation and soil moisture values were log or root transformed to meet the assumptions of
226 normality and homoscedasticity, while tables and figures show untransformed data. We also
227 calculated pairwise Pearson correlations between the measurements of RWC and soil moisture
228 that were taken at the same day (six measuring dates), considering all treatments together.
229 Survival analyses were run with R version 3.4.0 (R Core Team, 2017) and the survival
230 (Therneau, 2015) and survminer (Kassambara and Kosinski, 2017) packages, while the
231 ANOVAs were run with SPSS v19.0.

232 **3. Results**

233 **3.1. Seedling survival**

234 The survival rate was high at the end of GS2 (83% in the S-facing and 86% in the N-facing
235 trial), but dropped dramatically in GS3 (34% and 48% respectively). In the sub-experiment 1
236 the effect of mulches on seedling survival was not significant, with a single exception:
237 New_Jute resulted in a significantly higher survival rate (48%) than Com_Plastic (17%), but
238 only in the S-facing trial (Figure 1A). The use of soil conditioner had a positive effect in the N-
239 facing trial (57% survival with New_SC40 at the end of GS3, vs. 39% for SC-), but not in the S-
240 facing trial (Figure 1B). We found no significant interaction between mulches and soil
241 conditioner (data not shown). In sub-experiments 2 and 3 we could not detect any significant
242 effect of soil conditioner dosage or formulation in seedling survival (see Figures A1 and A2 in
243 Appendix A).



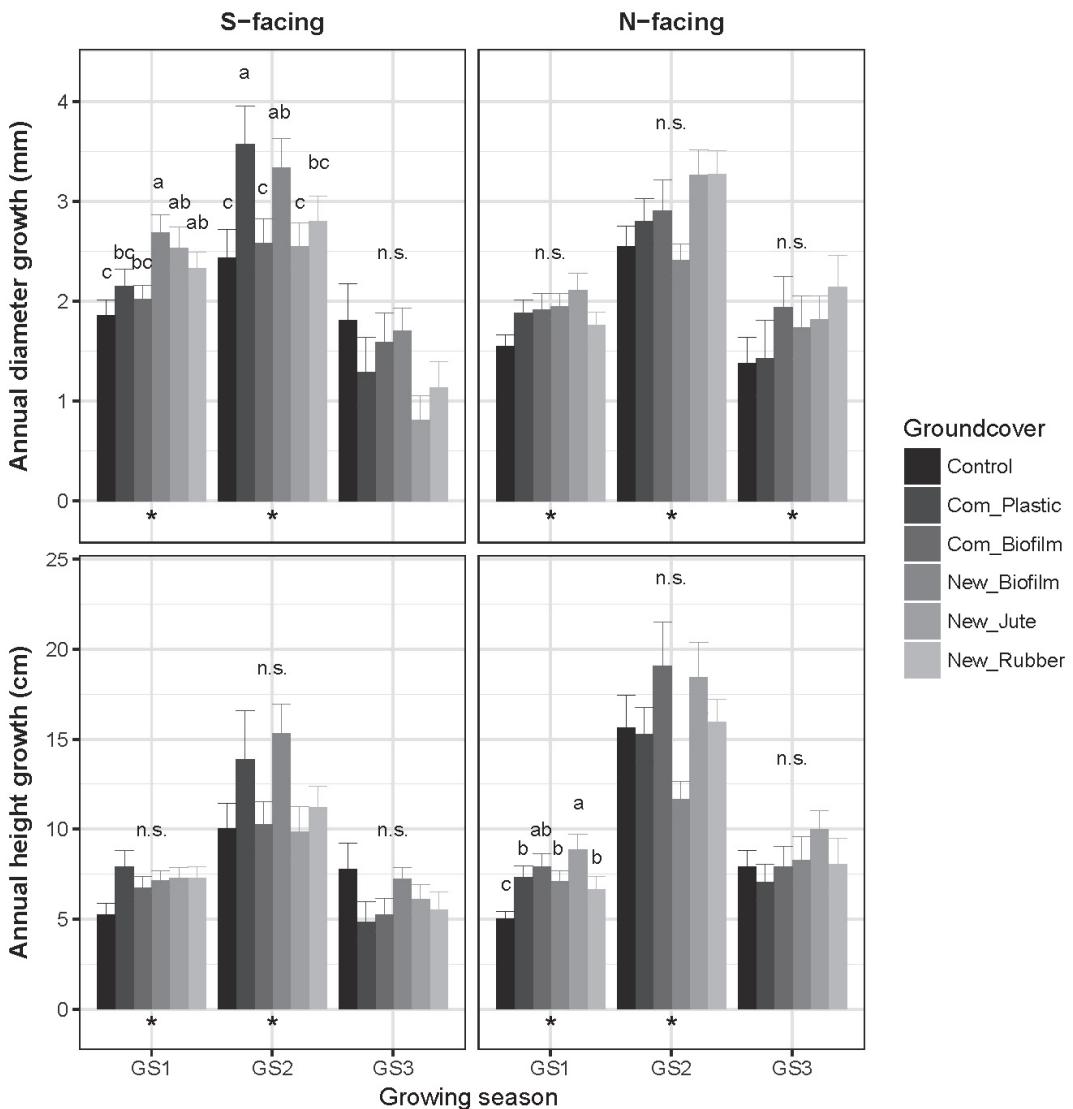
244

245 Figure 1. Survival curves based on Kaplan-Meier estimates for each field trial and factor of sub-
 246 experiment 1: combinations of (A) mulches and (B) the presence / absence of the new soil
 247 conditioner. Table 2 shows the complete description of each treatment.

248 3.2. Seedling growth

249 In sub-experiment 1 Control led to the lowest growth rates in most measurements, although the
250 differences with mulches were not always statistically significant. In N-facing trial all mulches
251 led to a significant increase of height growth compared to Control during GS1, while in S-
252 facing trial all mulches except for Com_Biofilm significantly increased the diameter growth
253 compared to Control seedlings in either GS1 and/or GS2 (Figure 2). We observed few
254 significant differences in growth as a result of mulch models: i) New_Biofilm led to higher
255 diameter growth than Com_Biofilm in GS1 and GS2 in S-facing trial and ii) New_Jute led to
256 higher height growth than Com_Plastic, New_Biofilm and New_Rubber in GS1 in N-facing
257 trial.

258 With regard to the use of soil conditioner, New_SC40 significantly increased (2-fold in average)
259 seedling diameter and height growth, compared to SC- in GS1 and GS2 and in both field trials.
260 In GS3 the positive effect of New_SC40 was noticeable in the diameter growth of N-facing trial
261 seedlings (Figure 2).



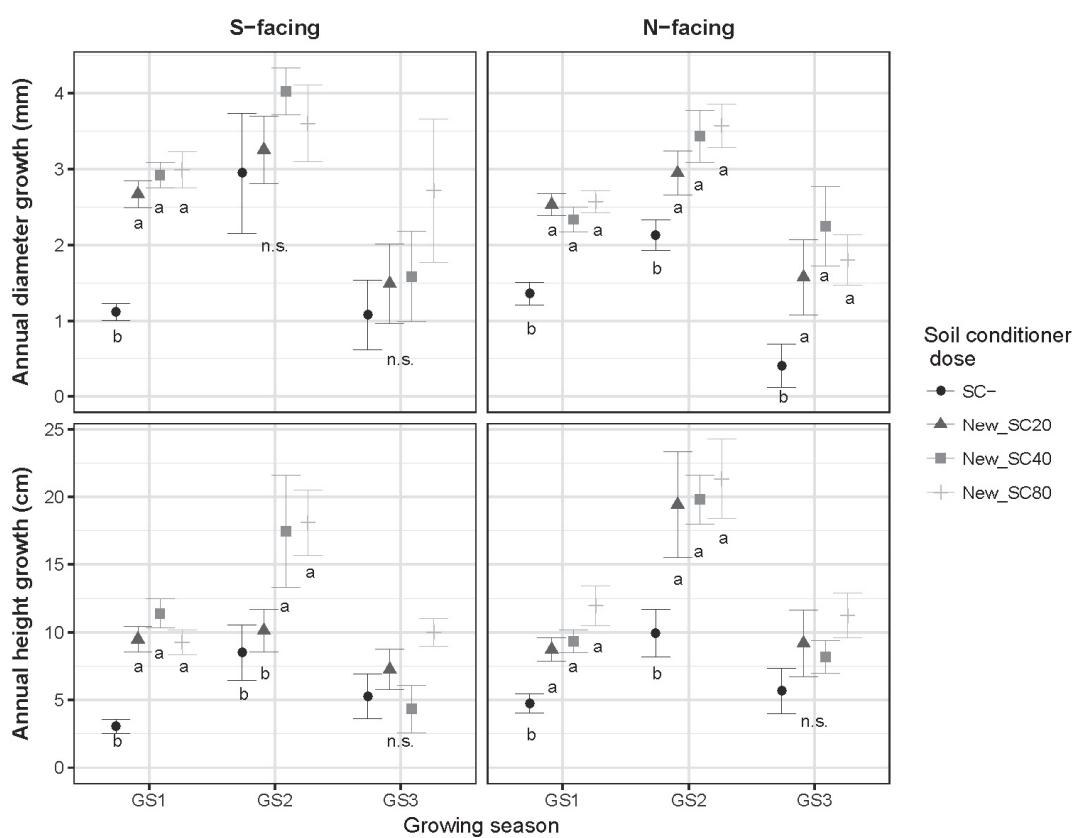
262

263 Figure 2. Diameter and height growth during the first growing seasons (GS1-GS3) for each
 264 mulch and presence of soil conditioner (sub-experiment 1), in both field trials. For each variable
 265 and year, significant differences ($p < 0.05$) between mulches are indicated by different letters
 266 (Duncan test grouping), while “n.s.” indicates lack of significance. The asterisks below the bars
 267 indicate significant growth increases induced by New_SC40 compared to SC-. Table 2 shows
 268 the description of each treatment.

269 The addition of soil conditioner at any dose (sub-experiment 2) resulted in significant increases
 270 in growth during the first two growing seasons. In general, height growth was positively related
 271 with the dosage, although there were no significant differences in growth between the dosages
 272 40 and 80 g seedling⁻¹ (Figure 3). In some cases, the dosage 20 g seedling⁻¹ did not yield
 273 significantly higher growth rates than SC-.

274 Both soil conditioner formulations (sub-experiment 3) led to similar growth rates (Tables B5
 275 and B6 in Appendix B). We only found a significantly lower height growth of seedlings treated
 276 with New_SC40 compared to Com_SC40 (9.34 ± 0.84 vs. 12.67 ± 0.94 ; $p = 0.011$) in the N-
 277 facing trial in GS1.

278 Appendix B shows the outcomes of the growth data analysis for each sub-experiment.



279

280 Figure 3. Diameter and height growth rates during the first three growing seasons (GS1-GS3 or
 281 2014-2016) for each field trial and soil conditioner dose (sub-experiment 2). Error bars
 282 represent standard errors. Significant differences ($p < 0.05$) between treatments for each
 283 variable and measuring date are indicated by different letters, grouped according to the Duncan
 284 test. No significant differences between treatments is indicated by “n.s.”. Table 2 shows the
 285 complete description of each treatment.

286 3.3. Biomass allocation

287 The results of sub-experiment 1 showed that adding soil conditioner (New_SC40) induced
 288 significant biomass gains for all fractions and in both trials (Table 3). This increase was greater
 289 for shoot mass than for root mass, leading to a significantly lower root:shoot ratio than SC-
 290 (0.43 ± 0.02 vs. 0.57 ± 0.03 in N-facing trial; 0.41 ± 0.01 vs. 0.56 ± 0.03 in S-facing trial; $p <$
 291 0.001 in both cases). On the other hand, we did not find any significant effect of mulching or of
 292 the interaction between mulch and soil conditioner on biomass allocation (Table C1 in
 293 Appendix C).

294 Table 3. Biomass allocation in GS1 (2014) in the two field trials, for seedlings with
 295 (New_SC40) and without (SC-) the new soil conditioner at $40\text{ g seedling}^{-1}$ dose (sub-experiment
 296 1). Value: mean \pm standard error of 36 samples. Significant differences ($p < 0.05$) between
 297 treatments are indicated by asterisks.

| | Root mass (g) | Shoot mass (g) | Total biomass (g) | Root:shoot ratio |
|-----------------------|-------------------|-------------------|-------------------|-------------------|
| S-facing trial | | | | |
| SC- | 1.81 ± 0.11 | 3.64 ± 0.35 | 5.45 ± 0.45 | 0.56 ± 0.03 |
| New_SC40 | $3.38 \pm 0.23^*$ | $8.75 \pm 0.72^*$ | $12.1 \pm 0.92^*$ | $0.41 \pm 0.01^*$ |
| N-facing trial | | | | |
| SC- | 1.55 ± 0.08 | 2.98 ± 0.22 | 4.53 ± 0.28 | 0.57 ± 0.03 |
| New_SC40 | $2.78 \pm 0.17^*$ | $6.84 \pm 0.58^*$ | $9.75 \pm 0.73^*$ | $0.43 \pm 0.02^*$ |

298

299 Higher dosages of the new soil conditioner (sub-experiment 2) led to significant increases in
 300 root, shoot and total mass in the S-facing trial, although the effect seemed to saturate at high
 301 doses: for example, total biomass (g seedling^{-1}) averaged 3.95 ± 0.61 (SC-); 8.15 ± 1.71
 302 (New_SC20); 14.1 ± 3.17 (New_SC40) and 14.9 ± 2.58 (New_SC80), and the difference
 303 between the two latter was not significant. The same pattern held true for root and shoot
 304 biomass. In the N-facing trial we found no significant differences in root, shoot or total biomass
 305 between the four dosages.

306 Finally, sub-experiment 3 did not lead to any significant difference between the different soil
 307 conditioner formulations on biomass allocation parameters.

308 Appendix C shows the outcomes of the biomass allocation analysis for each sub-experiment and
 309 field trial.

310

311 3.4. Seedling water status and soil moisture

312 In sub-experiment 1 we could not detect any significant effect of mulch on seedling RWC
313 (Table D1 in Appendix D). Conversely, all mulch models led to higher soil moisture than
314 control in N-facing trial at 20-40 cm depth ($p < 0.001$). However we did not find any significant
315 effect at 0-20 cm depth in this trial ($p = 0.701$) nor in S-facing trial whatsoever. Using soil
316 conditioner (New_SC40) resulted in significant increases in RWC as compared to SC- ($p =$
317 0.010; Table D1), and in higher soil moisture at 20-40 cm depth when considering both field
318 trials together ($p = 0.015$).

319 In sub-experiment 2, although we found no difference on RWC between the three tested
320 dosages (20, 40 and 80 g seedling⁻¹), all of them resulted in higher RWC value than SC- in both
321 N-facing and S-facing trials ($p < 0.001$ and $p = 0.028$; Table D2 in Appendix D). However, we
322 found no significant effect of the different soil conditioner dosages on soil moisture in any trial.

323 In sub-experiment 3, the new formulation resulted in a higher RWC value than the commercial
324 one, but the difference was only significant in the S-facing trial ($p = 0.025$; see Table D3).
325 Likewise, New_SC40 led to higher soil moisture than Com_SC40 at 20-40 cm depth (16.0 ± 0.5
326 vs. 14.4 ± 0.4 ; $p = 0.009$), but the opposite effect was observed at 0-20 cm depth (11.7 ± 0.5 vs.
327 13.9 ± 0.6 ; $p = 0.005$).

328 Overall we found a weak correlation between RWC and soil moisture. The r coefficients for the
329 0-20 cm soil depth were low, but significant: -0.36 ($p = 0.004$) for N-facing trial and -0.23 ($p =$
330 0.012) for S-facing trial. In the case of 20-40 cm depth the correlation was not significant in any
331 trial ($p = 0.257$; $p = 0.843$, respectively).

332 Appendix D shows the outcomes of RWC analyses.

333 **4. Discussion**

334 The hypotheses of sub-experiment 1 were partially corroborated: the benefits of mulching were
335 limited to punctual improvements in seedling growth and soil moisture, while the soil
336 conditioner had a positive effect in all seedling performance variables (survival, growth, water
337 status) and on soil moisture. The interaction of mulches and soil conditioner was not synergetic
338 as initially foreseen, but merely additive. The hypothesis of sub-experiment 2 was corroborated:
339 the improvements induced by the soil conditioner were in general proportional to the application
340 doses, although a saturating effect was detected, since we found no significant improvement of
341 applying 80 g seedling⁻¹ compared to 40 g seedling⁻¹. The hypothesis of sub-experiment 3 was
342 also verified, with a generally similar performance of New_SC40 and Com_SC40.

343 **4.1. Mulch performance**

344 The five tested mulches led to slight gains in seedling growth and soil moisture, compared to
345 control. Overall there was a 22% and 8% increase in mean diameter and height growth
346 respectively, in line with previous works using mulches with pine species (Blanco-García et al.
347 2011; McConkey et al. 2012). The positive effect of mulching on soil moisture at 20-40 cm was
348 in line with Valdecantos et al. (2009), who observed soil moisture increases under larger
349 mulches (0.35 m²), but in contrast with further results obtained by the same authors
350 (Valdecantos et al. 2014), who did not observe an increase under 40 x 40 cm mulches in another
351 semiarid site.

352 The lack of effect of mulching on seedling survival, biomass allocation or RWC may be due to
353 several reasons:

354 (i) the low site quality limited the proliferation of extant vegetation and therefore the weeding
355 benefits obtained by mulching. Even after three growing seasons weeds were nearly absent in
356 the unmulched planting pits, when we had foreseen a poor, yet active weed development;

357 (ii) our mulches (40 x 40 cm) were rather small compared to most previous works. Available
358 data on small mulches (60 x 60 cm or less) suggest that they result in slight gains in seedling
359 performance and micro-site conditions (Navarro et al. 2005; Dostálek et al. 2007; Valdecantos
360 et al. 2009, 2014), in contrast with the clearer benefits of units sized 80 x 80 cm or larger
361 (Jiménez et al. 2014, 2016; Coello et al. 2017);

362 (iii) conifers often show a more limited response to weed suppression than hardwoods (Van
363 Sambeek, 2010). Haywood (2000) and McConkey et al. (2012) even failed to detect positive
364 effects of mulches on pine survival.

365 While we tested a range of mulch models with various technical, economic and environmental
366 implications, eventually all of them led to similar effects on the variables measured, in line with
367 Johansson et al. (2006) and Maggard et al. (2012). Among the five tested models the best
368 overall performance could be attributed to New_Jute, which ranked first on overall seedling
369 survival, height growth, shoot and total biomass and RWC, although the improvement
370 compared to other mulch models was seldom statistically significant. The pale color of this
371 model (thus not accumulating heat) and its permeability (allowing water infiltration in the case
372 of light rainfall episodes) might have been advantages compared to alternative models in this
373 study area subject to high temperatures and light precipitation episodes.

374 4.2. Soil conditioner performance

375 The new soil conditioner applied at 40 g seedling⁻¹ (sub-experiment 1) had beneficial effects on
376 seedling survival, growth, water status and soil moisture, compared to the control (non-
377 application). In the N-facing trial the survival rate of seedlings with New_SC40 (almost 60%)
378 was higher than that of control seedlings (37%), which were similar to the overall survival in the
379 S-facing trial (35%) and to most studies in Semiarid or in low quality Mediterranean conditions
380 with Aleppo pine: 31% (Del Campo et al. 2007a), 7-44% (Del Campo et al. 2007b) and 10-50%
381 (Fuentes et al. 2010).

382 Diameter and height growth gains induced by New_SC40 compared to SC- were significant,
383 achieving better results than field studies testing pure SAP as soil conditioner (Clemente et al.
384 2004; Barberà et al. 2005; Chirino et al. 2011). Compared to SC-, seedlings treated with
385 New_SC40 had 80% higher root mass and 135% higher shoot mass during the first growing
386 season. Contrary to our results, nursery studies with Aleppo pine show that SAPs induce larger
387 gains in root mass than in shoot mass components (Hüttermann et al. 1999; Del Campo et al.
388 2011).

389 New_SC40 also had an overall positive effect on seedling water status and soil moisture, as
390 observed both in field experiments (Clemente et al. 2004) and in nursery conditions (Beniwal et
391 al. 2011; Chirino et al. 2011). The improvement in water availability induced by soil
392 conditioners has been linked to the reduction in evaporative and percolation losses, especially in
393 coarse-textured soils as the one in our study (Koupai et al. 2008; Del Campo et al. 2011). This
394 improvement may be behind the enhanced survival and growth rates observed for this treatment.
395 However, in our trials, the presence of seedlings using available water makes it difficult to
396 extract more definitive conclusions about the effect of treatments on soil moisture and its
397 consequences on seedling performance. Keeping some experimental plots without seedlings

398 (Arbona et al. 2005) and recording soil moisture on a continuous basis can shed more light on
399 the interpretation of this variable.

400 Several field studies in Mediterranean conditions have observed losses of the soil conditioner
401 effect after few growing seasons (Chirino et al. 2011; Oliveira et al. 2011). This agrees with our
402 results: in our case, the effects almost disappeared after the second growing season, which may
403 be due to three possible and complementary reasons:

404 (i) once the root system develops out of the plantation pit, the new roots grow in non-
405 conditioned soil and cannot benefit from the effect of this technique;

406 (ii) the swelling capacity of SAP may decrease over time. In this regard, Holliman et al. (2005)
407 observed this limitation after 18 months, although this figure may vary with the particular
408 polymer/s;

409 (iii) the drought severity in GS3 may have transcended the new soil conditioner's capacity to
410 help the seedling withstand the water deficit. The summer rainfall during GS3 was only 11 mm
411 (one sixth of the historical average) and led to a dramatic decrease in overall survival rate (85%
412 to 41%). In this regard, Del Campo et al. (2011) observed that the soil conditioner increased
413 RWC and soil moisture under moderately dry conditions (few weeks after a rainfall), but not in
414 severely dry ones (drought extended for several months). The low number of measuring dates
415 did not allow us to draw definitive conclusions in this respect and future research should aim to
416 detect the threshold drought intensity at which the effectiveness of a soil conditioner decreases.

417 4.3. Soil conditioner dosage and formulation

418 Increasing the dosage of the New_SC (sub-experiment 2) beyond the manufacturer's
419 recommendation ($40 \text{ g seedling}^{-1}$) did not result in significant improvements in plant
420 performance. This prescribed dose of the New_SC represents 14.5 g of SAP or 0.02% in weight
421 when applied at $30 \times 30 \times 30 \text{ cm}$ soil volume, which is five times less than the SAP dosage
422 recommended by Del Campo et al. (2011) for sandy and loamy-sandy soils.

423 Sub-experiment 3 showed that the new polyacrylamide-free formulation (New_SC) performed
424 similarly to the commercial one (Com_SC) at the same dose for most variables measured and
425 can be therefore considered as a suitable alternative. Both SCwSAPs (New_SC and Com_SC)
426 outperformed the results obtained with pure SAP by Clemente et al. (2004), who found that 100
427 g seedling^{-1} dose had no effect on seedling performance. This better performance of SCwSAP
428 compared to pure SAP at higher doses could be related to its synergistic mixture of SAP with
429 other components (fertilizers, humic acids, root growth precursors), as suggested in nursery

430 conditions (Vieira et al. 2005; Machado et al. 2016). Future field studies could assess the effect
431 of each SCwSAP on seedling and soil nutrient status.

432 **4.4. Implications for management**

433 The new soil conditioner, especially when applied at a dose of 40 g seedling⁻¹, improved early
434 seedling performance at a site severely limited by a low precipitation and a coarse textured soil.
435 The main strengths of this technique, as compared to support irrigation, are cost-effectiveness,
436 water saving during dry periods, easy application done during plantation and the lack of tending
437 required (Coello and Piqué, 2016). It is also expected to have higher social acceptability than
438 most commercially available soil conditioners including SAP with polyacrylamide (DRI, 2008).
439 Nevertheless, its effect seems to be limited to a few years and to moderate drought events.
440 Further research should help elucidate the extent of these limitations.

441 Compared to soil conditioners, small mulches only produced slight benefits in seedling
442 performance, making us conclude that this should not be a priority technique in pine
443 afforestation in semiarid conditions. Wherever mulching is a suitable technique, the three new
444 prototypes, which are either biodegradable (New_Biofilm, New_Jute) or made of recycled
445 waste (New_Rubber) induced very similar performance than the reference plastic mulch,
446 making them added-value alternatives considering technical, social and environmental aspects.

447 Our study is the first field test for the new soil conditioner and the second one including the new
448 mulch prototypes (see Vitone et al. 2016). However, we tested these techniques on a single tree
449 species at only two sites, and thus caution should be taken before generalizing the conclusions
450 to other conditions. Further research examining the single and combined effect of mulches and
451 soil conditioners in different environmental conditions and for different plant species may help
452 elucidate their potential as restoration tools from the technical, economic and environmental
453 points of view.

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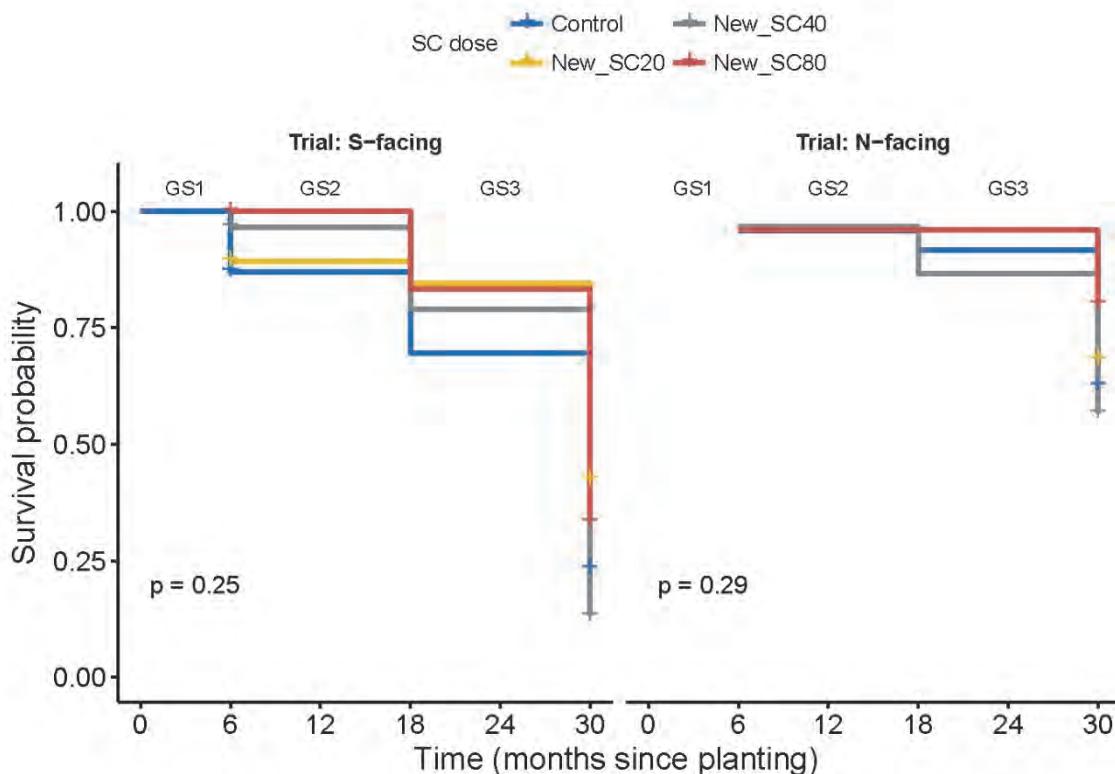
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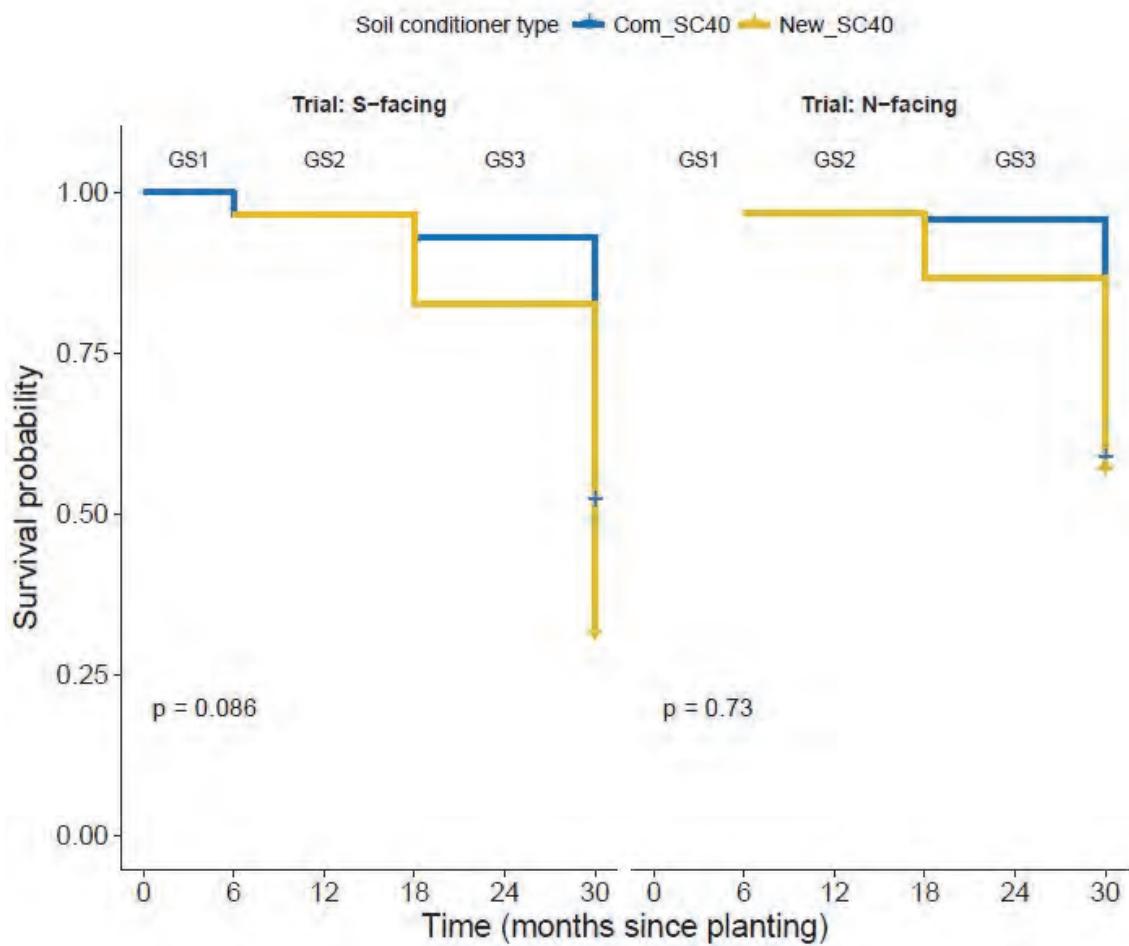
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614 **Appendices**

615 **Appendix A. Survival rates of sub-experiments 2 (soil conditioner dosage) and 3**
616 **(soil conditioner formulation)**



618 Figure A1. Survival rate in G1-GS3 in both field trials, for trees without (SC-) and with
619 various doses of the new soil conditioner (20, 40 and 80 g seedling⁻¹), combined with a
620 plastic mulch (Com_Plastic), sub-experiment 2.



621

622 Figure A2. Survival rate in G1-GS3 in both field trials, for trees with the commercial
623 (Com_SC) and the new (New_SC) soil conditioners, both applied at 40 g seedling⁻¹ and
624 combined with a plastic mulch (Com_Plastic), sub-experiment 3.

625 **Appendix B. Tree growth results**

626 Sub-experiment 1 (factorial design)

627 Table B1. Results of the two-way ANOVA on the effect of the mulches and the new
 628 soil conditioner, as well as their interaction (sub-experiment 1) on seedling diameter
 629 growth (DG) per growing season (GS).

| | DG GS1 | | | DG GS2 | | | DG GS3 | | |
|--------------------------|--------|----|---------|--------|----|---------|--------|----|---------|
| | F | dF | p-value | F | dF | p-value | F | dF | p-value |
| S-facing trial | | | | | | | | | |
| Soil conditioner | 208.15 | 5 | <0.001 | 96.08 | 5 | <0.001 | 0.14 | 5 | 0.729 |
| Mulch | 6.02 | 1 | 0.001 | 4.01 | 1 | 0.007 | 1.98 | 1 | 0.155 |
| Soil conditioner * mulch | 0.436 | 5 | 0.819 | 0.43 | 5 | 0.823 | 1.03 | 5 | 0.503 |
| N-facing trial | | | | | | | | | |
| Soil conditioner | 127.66 | 5 | <0.001 | 20.86 | 5 | 0.006 | 17.70 | 5 | 0.008 |
| Mulch | 2.08 | 1 | 0.103 | 1.78 | 1 | 0.151 | 0.92 | 1 | 0.482 |
| Soil conditioner * mulch | 2.00 | 5 | 0.116 | 1.52 | 5 | 0.220 | 0.858 | 5 | 0.540 |

630

631 Table B2. Results of the two-way ANOVA on the effect of the mulches and the new
 632 soil conditioner, as well as their interaction (sub-experiment 1) on seedling height
 633 growth (HG) per growing season (GS).

| | HG GS1 | | | HG GS2 | | | HG GS3 | | |
|--------------------------|--------|----|---------|--------|----|---------|--------|----|---------|
| | F | dF | p-value | F | dF | p-value | F | dF | p-value |
| S-facing trial | | | | | | | | | |
| Soil conditioner | 119.96 | 5 | <0.001 | 75.08 | 5 | <0.001 | 2.78 | 5 | 0.135 |
| Mulch | 1.39 | 1 | 0.262 | 0.937 | 1 | 0.474 | 1.02 | 1 | 0.433 |
| Soil conditioner * mulch | 0.382 | 5 | 0.856 | 1.37 | 5 | 0.273 | 0.372 | 5 | 0.853 |
| N-facing trial | | | | | | | | | |
| Soil conditioner | 70.85 | 5 | <0.001 | 129.99 | 5 | <0.001 | 2.11 | 5 | 0.196 |
| Mulch | 4.16 | 1 | 0.007 | 2.01 | 1 | 0.112 | 0.746 | 1 | 0.596 |
| Soil conditioner * mulch | 4.15 | 5 | 0.008 | 3.07 | 5 | 0.027 | 0.642 | 5 | 0.673 |

634

635 Sub-experiment 2 (dosage of new soil conditioner)

636 Table B3. Results of the one-way ANOVA on the effect of the dosage of the new soil
 637 conditioner (sub-experiment 2) on seedling diameter growth (DG) per growing season
 638 (GS).

| | F | DG GS1 dF | p-value | F | DG GS2 dF | p-value | F | DG GS3 dF | p-value |
|------------------------------|-------|--------------|---------|------|--------------|---------|------|--------------|---------|
| S-facing trial | | | | | | | | | |
| Dose of new soil conditioner | 23.75 | 3 | <0.001 | 0.78 | 3 | 0.511 | 0.77 | 3 | 0.528 |

639

640 Table B4. Results of the one-way ANOVA on the effect of the dosage of the new soil
 641 conditioner (sub-experiment 2) on seedling height growth (HG) per growing season
 642 (GS).

| | F | HG GS1 dF | p-value | F | HG GS2 dF | p-value | F | HG GS3 dF | p-value |
|------------------------------|-------|--------------|---------|-------|--------------|---------|------|--------------|---------|
| S-facing trial | | | | | | | | | |
| Dose of new soil conditioner | 18.07 | 3 | <0.001 | 6.617 | 3 | 0.001 | 2.54 | 3 | 0.089 |

643

644 Sub-experiment 3 (formulation of new soil conditioner)

645 Table B5. Results of the ANOVA on the effect of soil conditioner type (sub-experiment
 646 3) on seedling diameter growth (DG) per growing season (GS).

| | DG GS1 | | | DG GS2 | | | DG GS3 | | |
|--------------------------|--------|----|---------|--------|----|---------|--------|----|---------|
| | F | dF | p-value | F | dF | p-value | F | dF | p-value |
| S-facing trial | | | | | | | | | |
| Type of soil conditioner | 0.06 | 1 | 0.814 | 4.20 | 1 | 0.05 | 0.35 | 1 | 0.565 |
| N-facing trial | | | | | | | | | |
| Type of soil conditioner | 1.54 | 1 | 0.220 | 0.30 | 1 | 0.588 | 0.47 | 1 | 0.503 |

647

648 Table B6. Results of the one-way ANOVA on the effect of soil conditioner type (sub-
 649 experiment 3) on seedling height growth (HG) per growing season (GS). DF=1

| | HG GS1 | | | HG GS2 | | | HG GS3 | | |
|--------------------------|--------|----|---------|--------|----|---------|--------|----|---------|
| | F | dF | p-value | F | dF | p-value | F | dF | p-value |
| S-facing trial | | | | | | | | | |
| Type of soil conditioner | 0.16 | 1 | 0.691 | 0.08 | 1 | 0.781 | 2.45 | 1 | 0.149 |
| N-facing trial | | | | | | | | | |
| Type of soil conditioner | 6.92 | 1 | 0.011 | 0.04 | 1 | 0.842 | 0.57 | 1 | 0.459 |

650

651 **Appendix C. Biomass allocation**

652 Sub-experiment 1 (factorial design)

653 Table C1. Results of the two-way ANOVA on the effect of the mulches and the new
 654 soil conditioner, as well as their interaction (sub-experiment 1) on various biomass
 655 allocation components, during GS1 (2014).

| | Root mass (g) | | | Shoot mass (g) | | | Total mass (g) | | | Root:shoot ratio | | |
|-----------------------|------------------|----|---------|-------------------|----|---------|-------------------|----|---------|------------------|----|---------|
| | F | dF | p-value | F | dF | p-value | F | dF | p-value | F | dF | p-value |
| S-facing trial | | | | | | | | | | | | |
| Mulch | 0.32 | 5 | 0.901 | 1.16 | 5 | 0.340 | 0.93 | 5 | 0.470 | 1.72 | 5 | 0.143 |
| Soil conditioner | 50.56 | 1 | <0.001 | 64.10 | 1 | <0.001 | 62.97 | 1 | <0.001 | 21.90 | 1 | <0.001 |
| Mulch * | 0.95 | 5 | 0.457 | 1.24 | 5 | 0.304 | 0.85 | 5 | 0.521 | 1.81 | 5 | 0.124 |
| soil conditioner | | | | | | | | | | | | |
| N-facing trial | | | | | | | | | | | | |
| Mulch | 0.29 | 5 | 0.919 | 0.36 | 5 | 0.873 | 0.31 | 5 | 0.907 | 0.63 | 5 | 0.680 |
| Soil conditioner | 48.29 | 1 | <0.001 | 54.67 | 1 | <0.001 | 60.94 | 1 | <0.001 | 14.30 | 1 | <0.001 |
| Mulch * | 0.60 | 5 | 0.701 | 0.81 | 5 | 0.546 | 0.77 | 5 | 0.578 | 0.50 | 5 | 0.772 |
| soil conditioner | | | | | | | | | | | | |

656

657 Sub-experiment 2 (dosage of new soil conditioner)

658 Table C2. Results of the ANOVA on the effect of the dosage of the new soil conditioner
 659 (sub-experiment 2) on various biomass allocation components, during GS1 (2014).

| | Root mass (g) | | | Shoot mass (g) | | | Total mass (g) | | | Root:shoot ratio | | |
|------------------------------|------------------|----|---------|-------------------|----|---------|-------------------|----|---------|------------------|----|---------|
| | F | dF | p-value | F | dF | p-value | F | dF | p-value | F | dF | p-value |
| S-facing trial | | | | | | | | | | | | |
| Dose of new soil conditioner | 5.17 | 3 | 0.008 | 9.60 | 3 | <0.001 | 9.60 | 3 | <0.001 | 8.40 | 3 | 0.001 |
| N-facing trial | | | | | | | | | | | | |
| Dose of new soil conditioner | 3.35 | 3 | 0.042 | 3.90 | 3 | 0.026 | 3.99 | 3 | 0.024 | 1.18 | 3 | 0.345 |

660

661 Sub-experiment 3 (formulation of new soil conditioner)

662 Table C3. Results of the ANOVA on the effect of soil conditioner formulation (sub-
 663 experiment 3) on various biomass allocation components, during GS1 (2014).

| | Root mass (g) | | | Shoot mass (g) | | | Total mass (g) | | | Root:shoot ratio | | |
|--------------------------|------------------|----|---------|-------------------|----|---------|-------------------|----|---------|------------------|----|---------|
| | F | dF | p-value | F | dF | p-value | F | dF | p-value | F | dF | p-value |
| S-facing trial | | | | | | | | | | | | |
| Type of soil conditioner | 0.01 | 1 | 0.911 | 0.42 | 1 | 0.530 | 0.30 | 1 | 0.594 | 0.65 | 1 | 0.440 |
| N-facing trial | | | | | | | | | | | | |
| Type of soil conditioner | 2.39 | 1 | 0.157 | 1.73 | 1 | 0.221 | 2.04 | 1 | 0.187 | 0.02 | 1 | 0.894 |

664

665 **Appendix D. Needle relative water content (RWC)**

666 Sub-experiment 1 (factorial design)

667 Table D1. Results of the two-way ANOVA on the effect of the use of mulch and the
 668 new soil conditioner, as well as their interaction (sub-experiment 1) on seedling
 669 Relative needle Water Content (RWC), measured six times in 2014 and 2015: July GS1
 670 (2 measurements), August GS1, September GS1, July GS2 and August GS2.

| | F | RWC dF | p-value | |
|--------------------------|-------|-----------|---------|--|
| S-facing trial | | | | |
| Mulch | 2.16 | 5 | 0.057 | |
| Soil conditioner | 26.49 | 1 | <0.001 | |
| Mulch * soil conditioner | 2.91 | 5 | 0.013 | |
| N-facing trial | | | | |
| Mulch | 0.77 | 5 | 0.569 | |
| Soil conditioner | 6.70 | 1 | 0.010 | |
| Mulch * soil conditioner | 3.76 | 5 | 0.002 | |

671 Sub-experiment 2 (dosage of new soil conditioner)

672 Table D2. Results of the one-way ANOVA on the effect of the dosage of the new soil
 673 conditioner (sub-experiment 2) on seedling Relative needle Water Content (RWC),
 674 measured six times in 2014 and 2015: July GS1 (2 measurements), August GS1,
 675 September GS1, July GS2 and August GS2.

| | F | RWC dF | p-value | |
|------------------------------|------|-----------|---------|--|
| S-facing trial | | | | |
| Dose of new soil conditioner | 8.53 | 3 | <0.001 | |
| N-facing trial | | | | |
| Dose of new soil conditioner | 3.11 | 3 | 0.028 | |

676 Sub-experiment 3 (formulation of new soil conditioner)

677 Table D3. Results of the one-way ANOVA on the effect of soil conditioner type (sub-
 678 experiment 3) on seedling Relative needle Water Content (RWC), measured six times in
 679 2014 and 2015: July GS1 (2 measurements), August GS1, September GS1, July GS2
 680 and August GS2.

| | F | RWC dF | p-value | |
|--------------------------|------|-----------|---------|--|
| S-facing trial | | | | |
| Type of soil conditioner | 5.22 | 1 | 0.025 | |
| N-facing trial | | | | |
| Type of soil conditioner | 3.07 | 1 | 0.083 | |

681

Anexo 4. Artículo correspondiente al Ensayo 4.



RESEARCH ARTICLE

OPEN ACCESS

Combining innovative mulches and soil conditioners in mountain afforestation with ash (*Fraxinus excelsior* L.) in the Pyrenees (NE Spain)

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Abstract

Aim of study: To assess the effectiveness for improving early seedling performance of the individual and combined application of (i) various doses of an innovative soil conditioner including polyacrylamide-free super-absorbent polymers, fertilizers, root precursors and humic acids; and (ii) innovative mulches based on renewable-biodegradable or recycled raw materials. The assessment was carried out in comparison with reference (commercial) soil conditioners and mulches.

Area of study: Upper montane afforestation site located at 1,430 m altitude in the southern Pyrenees (NE Spain).

Material and methods: We studied the effect of 15 treatments (various combinations of soil conditioners and mulches) on mountain ash (*Fraxinus excelsior* L.), testing survival, diameter and height growth and water and nutrient status during two growing seasons (2014–2015). We also assessed mulch durability during 2014–2016.

Main results: The innovative soil conditioner improved diameter and height seedling growth (92% and 72% respectively) and water and nutrient status. The 40 g/seedling dosage was more cost-effective than the 20 and 80 g/seedling doses. The new formulation performed better in general than the commercial formulation. Mulches led to slight gains compared to control seedlings, and there were no major differences between the mulch models. The combined application of soil conditioners and mulches was not of particular interest.

Research highlights: Soil conditioners consisting of synergic mixtures of water super-absorbent polymers, fertilizers, root growth precursors and humic acids can improve early seedling performance in coarse-textured, stony soils in montane conditions. Small mulches may be only of limited interest as long as weed competitiveness is poor.

Additional keywords: ecotechnology; groundcovers; reforestation; seedling performance; restoration; water super-absorbent polymer; weed.

Abbreviations used: GS_n, growing season number ‘n’; leaf water potential, LWP; water super-absorbent polymers, SAP; soil conditioner with water super-absorbent polymers, SCwSAP; Soil Plant Analysis Development, SPAD.

Authors' contributions: Conceived and designed the experiments: JC, PR and MP; data acquisition and management: CF and JC; critical revision of the manuscript for important intellectual content: JC, PR, AA and MP; coordinating the research project: MP.

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Supplementary material (Tables S1 and S2) accompany the paper on FS's website.

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Introduction

The history of land use in the Pyrenees, as in other mountain areas of south Europe, spreads over millennia. In the last century the modernization of farming in lowlands resulted in the abandonment of

many traditional agricultural and grassland practices (MacDonald *et al.*, 2000), particularly in the difficult to access, small-sized fields predominant in mountain areas. Consequently, forest has expanded significantly, especially pine forests (Ameztegui *et al.*, 2010). This has homogenized the landscape both in terms of

composition and structure. Afforestation with broadleaf trees helps to decrease the negative consequences of excessive landscape homogenization, such as an increase in fire risk (Palmero-Iniesta *et al.*, 2017) and negative effects on biodiversity (Ameztegui *et al.*, 2018), among others.

In the Pyrenees, European ash (*Fraxinus excelsior* L.) is a native broadleaf tree with many uses, including timber for tool-making and turnery, fodder (green branches and litter), charcoal and fuelwood (Marie-Pierre *et al.*, 2006; Mottet *et al.*, 2007). Despite these uses, many ash forests were cut down and turned into grasslands in the past (Vigo *et al.*, 2005). However, more recently, its ecological plasticity together with its fast growth and valuable timber has turned ash into a good candidate for afforestation programmes in Europe (Fraxigen, 2005; Weber-Blaschke *et al.*, 2008). The main limiting factor for ash in the southern Pyrenees is its sensitivity to water shortage (Gonin *et al.*, 2013), which is particularly critical during the first years when the root system is not yet well developed (Vallejo *et al.*, 2012). South European montane conditions are characterized by a dry season that coincides with the highest temperatures, and by shallow, coarse-textured soils with high stoniness and steep slopes, resulting in a poor water retention capacity. Soil water content can be increased by support irrigations, which are expensive and often inapplicable in these conditions (Carminati *et al.*, 2010). An alternative option is to mix water super-absorbent polymers (SAPs or hydrogels) with the soil of the planting pit. These polymers can absorb and store up to 400 times their weight in water (Bouranis *et al.*, 1995), which is then available to the plants over an extended time period (Hüttermann *et al.*, 2009). SAPs can increase soil water content by reducing evaporation and percolation losses, which is of particular interest in soils with a poor water retention capacity, i.e. coarse-textured soils (Bhardwaj *et al.*, 2007; Koupai *et al.*, 2008; Del Campo *et al.*, 2011). SAPs have been reported to alleviate soil and plant water potential, and increase plant water use efficiency and the time to reach permanent wilting point, ultimately enhancing plant survival and growth (Sivapalan, 2001; Hüttermann *et al.*, 2009; Del Campo *et al.*, 2011). SAPs are commercialized alone or as synergistic mixtures with other ingredients, especially fertilizers and various organic compounds, with the intention of improving both the physical and chemical soil properties and not only the water-related parameters. Moreover, SCwSAPs aim to prevent some of the limitations of pure SAPs, such as the reduction of NO_3^- and NH_4^+ availability and the risk of being washed away (Rowe *et al.*, 2005). These mixtures are regarded as soil conditioners with SAP (SCwSAP), and have been reported to enhance soil nutrient levels and

seedling performance (Machado *et al.*, 2016; Coello *et al.*, 2018). However, the application of SAPs and SCwSAPs at afforestation sites has obtained contrasting results depending on their composition, application method, dosage and soil features (Navarro *et al.*, 2005; Del Campo *et al.*, 2011; Coello *et al.*, 2018).

Despite their interest, there are some constraints involved in using SAP or SCwSAP, as most formulations are based on cross-linked polyacrylamide, which causes social concern due to the residual presence of non-polymerized acrylamide. Although the concentrations are within the legal limits (Rowe *et al.*, 2005) and acrylamide has a short half-life in the soil (Lande *et al.*, 1979), SAP manufacturers are currently developing polyacrylamide-free versions that still need to be tested in the field and compared to current commercial formulations. Moreover, a limiting factor of SAPs is that the improvement in conditions at micro-site level is positive for the seedling but also for the competing vegetation, which is a major threat to young afforestations (Willoughby *et al.*, 2009). Extant vegetation can outcompete newly established seedlings in the struggle for water, light and nutrients (Navarro-Cerrillo *et al.*, 2005). The most widespread weeding techniques are to apply herbicide, which is cost-effective but is of growing social concern (Willoughby *et al.*, 2009), and mechanical weeding, which is expensive and can only be applied at sites that can be accessed easily.

An alternative option is to use mulches, also known as groundcovers or weeding mats. A mulch is an opaque layer covering the soil around the seedling, impeding weeds from germinating in its vicinity (Maggard *et al.*, 2012). Previous works have demonstrated that mulching has positive effects on seedling survival and growth (Van Sambeek, 2010; Maggard *et al.*, 2012, Coello *et al.*, 2017), increases soil water content because it reduces evaporation and transpiration by weeds (Hueso-González *et al.*, 2015), improves nutrient uptake (Van Sambeek & Garrett, 2004) and buffers soil temperatures (Cregg *et al.*, 2009; Coello *et al.*, 2017). Although plastic mulches are the most widespread (Arentoft *et al.*, 2013), several new environmentally friendly mulch materials are being developed, including biofilms, i.e. plastic-like materials made from renewable sources (Kapanen *et al.*, 2008). Other similar materials include composites made from paper residues (Shogren & Rousseau, 2005) and jute tissues (Debnath, 2014).

Despite the interest in these new materials and techniques for afforestation, the combined application of soil conditioners and groundcovers has rarely been tested in field conditions (Navarro *et al.*, 2005; Coello *et al.*, 2018). This study aims to assess the effectiveness on

early seedling performance (survival, growth and water and nutrient status) of the individual and combined application of (a) new mulches based on renewable or recycled raw materials, either biodegradable or reusable; and (b) polyacrylamide-free SCwSAP, in south European montane conditions. We also studied mulch durability. We compared the performance of these new techniques with commercially available mulches and SCwSAP.

Our working hypotheses were: i) both the use of mulches and SCwSAP will have a positive effect on all seedling parameters compared to their respective controls, while the combined use of the two techniques will lead to a synergistic increase in performance; (ii) the performance of SCwSAP will be proportional to the application dosage; and (iii) the commercial (including polyacrylamide) and the new SCwSAP (polyacrylamide-free) will perform similarly when applied at the same dosage.

Materials and Methods

Description of the study area

The experiment was conducted in Fontanals de Cerdanya, in the Pyrenean mountains of Catalonia, NE Spain ($42^{\circ}23'9.11N$; $1^{\circ}55'53.90E$). The plot is located at a mean altitude of 1,430 m a.s.l, on a north aspect with an average slope inclination of 30%. The mean annual temperature is $7.5^{\circ}C$ while the mean annual precipitation

is 887 mm, 272 mm of which occur in summer. According to the Köppen classification, the climate is between Cfc (Temperate) and Dfb (Continental). The soil has a loamy-sandy texture (22% clay, 21% silt, 57% sand), the pH is 5.2 and organic matter content is 2.56%. Most soils in this area are Humic Dystrudepts (USDA, 1999) with a mesic temperature regime (Poch & Boixadera, 2008).

This plot was used for cattle grazing until it was abandoned in 2013. The main woody vegetation species, with a very low density, are the trees ash (*Fraxinus excelsior*) and wild pear (*Pyrus communis* L.), and the shrubs *Rosa canina* L. and *Rubus idaeus* L.

The annual summer precipitation was monitored continually with a weather station (Davis Instruments, USA). The summer of the first growing season (2014, GS1 hereinafter) was rather wet (404 mm) compared to the historical reference (272 mm, Ninyerola *et al.*, 2005), while the summer of the second growing season (2015, GS2 hereinafter) was drier (220 mm).

Experimental design and treatments

We planted 450 seedlings following a randomized incomplete block design with six blocks. In each block we planted 75 seedlings that were randomly assigned, in groups of 5 seedlings, to the 15 experimental treatments or combinations of various mulching and soil conditioning techniques. In total each treatment was applied to 30 seedlings. Table 1 shows the details of the techniques applied (mulch and soil conditioner).

Table 1. Description of the experimental techniques.

| Technique type | Technique code | Description |
|------------------|------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Mulch | Control | No mulch applied. |
| | Com_Plastic | Commercial black polyethylene film, anti-UV treated, 80 µm thick. |
| | Com_Biofilm | Ökolys®, a commercial green biodegradable woven mat. |
| | New_Biofilm | Prototype of black biodegradable frame (biopolymer), fused to a black commercial biodegradable film based on PHA (polyhydroxyalkanoate), 80 µm thick, manufactured by Groencreatie and DTC. The frame is to make installation easier. |
| | New_Jute | Prototype of biodegradable woven jute mat treated with furan bio-based resin for increased durability, manufactured by La Zeloise NV. |
| | New_Rubber | Prototype of black layer made of recycled rubber, anti-UV treated, 1.5 mm thick to make fixation unnecessary, manufactured by EcoRub BVBA. |
| | | |
| Soil conditioner | SC- | No soil conditioner applied. |
| | New_SC20; New_SC40; New_SC80 | TerraCottem Arbor®, at the prototype stage when tested. Product developed for tree and shrub planting. Its formulation includes a new generation of polyacrylamide-free water super-absorbent polymers (36.25% of total weight), fertilizers (14.5%; NPK 3-1-7), humic acids (0.75%), growth precursors (0.25%) and volcanic rock (48.25%). The numbers 20, 40 and 80 indicate the dosage (g/seedling). |
| | Com_SC40 | TerraCottem Universal®, a commercially available soil conditioner with cross-linked polyacrylamide and polyacrylic acid polymers (39.50%), fertilizers (10.50%; NPK 9-2-11), growth precursors (0.25%) and volcanic rock (49.75%). The dosage was 40 g/seedling. |

The 15 treatments were organized into three sub-experiments:

(i) Sub-experiment 1: a full factorial design combining the 6 different mulch treatments with a new SCwSAP applied at a dose of 40 g/seedling (New_SC40) as well as without it (SC-). Overall there were 12 treatments with 30 seedlings per treatment, thus 360 seedlings in total. The soil conditioner dose of 40 g/seedling corresponds to the manufacturer's recommendation for the most similar commercial product available.

(ii) Sub-experiment 2: a study of the effect of four different doses of New_SC (0, 20, 40 and 80 g/seedling), combined with a reference mulch (Com_Plastic) in all cases. Each treatment comprised 30 seedlings, with a total of 120 seedlings in this sub-experiment.

(iii) Sub-experiment 3: a study comparing a commercial and a new SCwSAP (Com_SC vs. New_SC), both applied at a dosage of 40 g/seedling and combined with a reference mulch (Com_Plastic). Each treatment comprised 30 seedlings, with a total of 60 seedlings.

The combination Com_Plastic x New_SC40 was present in all three sub-experiments, while the combination Com_Plastic x SC- was present in sub-experiments 1 and 2.

Field trial establishment

We planted the seedlings in late March 2014, during vegetative dormancy. Soil preparation consisted in mechanical soil digging (40 x 40 x 40 cm) with a backhoe spider excavator, which was used to make micro-basins for runoff collection. The plantation frame was 3 x 3 m, for a density of 1,100 seedlings·ha⁻¹. The tree species chosen was mountain ash (*Fraxinus excelsior* L.) from the local provenance Central Pyrenees. The seedlings were one-year old and provided in 300 cm³ containers. They were 15–20 cm high and met the general seedling quality criteria (Cortina *et al.*, 2006). We applied the soil conditioner right before planting following the manufacturer's indications: we dug a sub-pit sized 30 x 30 x 30 cm, put half of the dosage at the bottom of the pit and mixed the other half with the soil used for filling up the pit when the seedlings were planted. We installed the mulches manually right after planting. We chose a small mulch size (40 x 40 cm, similar to the area of the planting pits), to limit costs and because we predicted that there would be low to intermediate weed proliferation during the first years. To protect the afforestation from browsing damage by roe deer (*Capreolus capreolus* L.) we installed a 1.3 m high perimeter fence consisting of four lines of barbed wire.

Assessment of seedling survival and growth

We assessed all seedlings at the time of planting (March 2014) and at the end of the first two growing seasons (October 2014 and 2015) to determine their survivorship, diameter and height. We conducted an additional visual assessment of survival eight weeks after the seedlings had been planted to remove any seedlings from the study that had died soon after planting as a result of poor seedling quality or careless planting (two seedlings in total). We measured seedling diameter at a painted, constant point, 4–5 cm above the ground, with a precision of 0.1 mm using a digital calliper. Seedling height was measured from the ground up to the highest bud, with a cm precision, using a measuring tape. Annual growth rates of alive seedlings were calculated as the difference between the measurement at the end of each growing season and the previous measurement.

Assessment of physiological traits

We measured two traits related to seedling performance: midday leaf water potential (LWP, hereinafter), which is a proxy of seedling water status; and leaf SPAD (Soil Plant Analysis Development), which is a proxy of seedling nutrient status (Djumaeva *et al.*, 2012). We measured LWP using a pressure chamber (Solfranc Technologies, Spain) with N₂ as the pressure gas. We measured the pressure (bars) at which the water within the leaf was ejected through the petiole. In both 2014 and 2015 we conducted 4 fortnightly measurements in July and August. On each of these 8 dates we sampled one leaf from one randomly chosen seedling per treatment and block (n = 6; 90 measures in total). These measurements were taken between 10:00 and 14:00 solar time, i.e., at the highest sun angle. We used a Minolta SPAD-502 instrument (Minolta Camera Co, Japan) to measure SPAD. The LWP and SPAD were measured on the same dates, except for the two August measurements in GS1 when SPAD could not be measured. In each SPAD sampling we measured one randomly chosen alive seedling per treatment and block (n = 6; 72 measurements in total). We calculated the average SPAD value of three leaves from each sampled seedling. Both LWP and SPAD values were obtained from sun-exposed, fully elongated and healthy leaves located in the upper third of the sampled seedling.

Mulch durability

The physical integrity of mulches was assessed visually in October 2016 after 30 months in the field. The mulches were assigned to a damage category

depending on the proportion of their surface that was damaged, either physically (torn) or due to weeds growing through and on the mulch layer: (i) intact (no damage); (ii) slight damage (1-25% damage); (iii) intermediate damage (26-50%); severe damage (>50%).

Statistical analyses

We analysed the data independently for each sub-experiment. We considered treatment as a fixed factor and block as a random factor. The LWP and SPAD data collected on different measuring dates were combined to build a more robust dataset.

Seedling survival and mulch durability were analysed with descriptive statistics. Seedling annual diameter and height growth, LWP and SPAD were assessed with an ANOVA, which was two-way in sub-experiment 1 (factors: mulch, soil conditioner, and their interaction) and one-way in sub-experiments 2 and 3. We used a significance level of 0.05 and assessed pairwise differences between treatments with the post-hoc Duncan's multiple range test. When necessary (seedling growth and LWP), values were log or root transformed to meet the ANOVA assumptions of normality and homoscedasticity. Tables and figures show untransformed data. The ANOVAs were run with SPSS v19.0 software.

Results

Seedling survival

The overall survival rate at the end of the first growing season (GS1) was 99%, dropping to 93% at the end of the second growing season (GS2). In sub-experiment 1 the lowest survival rate after two growing seasons corresponded to New_Rubber (82%) and the Control (90%), while the rest of the mulches showed survival rates above 93%. For the soil conditioner, New_SC40 showed a similar survival rate at the end of GS2 as SC- (93% and 90%, respectively). In sub-experiment 2 the treatments leading to the lowest survival rates after two growing seasons were SC- (90%) and New_SC80 (93%), while New_SC20 and New_SC40 resulted in 97% and 100%, respectively. In sub-experiment 3, both Com_SC40 and New_SC40 led to high survival rates at the end of GS2 (97% and 100%, respectively). Table S1 [suppl.] provides the annual survival of all treatments and sub-experiments.

Seedling growth

Table 2 shows the outcomes of the ANOVAs conducted in each sub-experiment. In sub-experiment 1 the mulches did not significantly affect annual diameter growth in GS1 or GS2; however, for height growth

Table 2. Summary of outcomes of the ANOVAs of annual diameter (DG) and height (HG) growth during the first (GS1, 2014) and the second (GS2, 2015) growing seasons; seedling water status (midday Leaf Water Potential, LWP) and seedling nutrient status (Soil Plant Analysis Development, SPAD).

| | DG GS1 | DG GS2 | HG GS1 | HG GS2 | LWP | SPAD |
|---------------------------------------------|--------|--------|-------------------------------------|--------|-------|-------|
| Sub-experiment 1 | | | | | | |
| | | | Mulch (dF=5) | | | |
| F | 1.02 | 1.13 | 2.07 | 2.92 | 1.25 | 1.46 |
| p-value | 0.409 | 0.344 | 0.072 | 0.020 | 0.284 | 0.203 |
| Soil conditioner (dF=1) | | | | | | |
| F | 201.41 | 8.88 | 30.592 | 1.29 | 6.10 | 4.49 |
| p-value | <0.001 | 0.003 | <0.001 | 0.261 | 0.014 | 0.035 |
| Interaction mulch * soil conditioner (dF=5) | | | | | | |
| F | 2.04 | 1.78 | 0.247 | 1.12 | 1.66 | 2.51 |
| p-value | 0.072 | 0.117 | 0.941 | 0.361 | 0.142 | 0.030 |
| Sub-experiment 2 | | | | | | |
| | | | Soil conditioner dose (dF=3) | | | |
| F | 28.67 | 4.79 | 7.73 | 1.76 | 0.06 | 3.71 |
| p-value | <0.001 | 0.004 | <0.001 | 0.185 | 0.979 | 0.013 |
| Sub-experiment 3 | | | | | | |
| | | | Soil conditioner formulation (dF=1) | | | |
| F | 6.85 | 8.63 | 3.02 | 0.07 | 0.85 | 8.05 |
| p-value | 0.011 | 0.005 | 0.092 | 0.799 | 0.360 | 0.006 |

New_Biofilm induced significantly higher growth rates than the Control in GS2 (Figure 1A). The use of soil conditioner resulted in a higher diameter and height growth than in the Control, although differences were greater for GS1 than for GS2, where differences between treatments were significant for diameter growth but not for height growth (Figure 1B). No significant effect of the interaction between mulch and soil conditioner on diameter growth or height growth was found (Table 2).

In sub-experiment 2 we observed that the diameter growth during GS1 was larger with increasing doses of soil conditioner, although the two highest doses (40 and 80 g/seedling) were not significantly different (Table 2). However, although the addition of soil conditioner also increased diameter growth during GS2, we did not observe significant differences between the tested doses (Figure 2A). In the case of height growth the pattern was unclear in GS1, with New_SC40 leading to higher values than New_SC20, which in turn was not significantly different to New_SC80. In GS2 there were no significant differences between treatments for height growth.

In sub-experiment 3 we observed significantly higher diameter growth when New_SC40 was used compared to Com_SC40 in both GS1 and GS2, while height growth rates were not significantly different (Figure 2B). Table S2 [suppl.] provides the annual growth values for each treatment.

Physiological traits

In sub-experiment 1 neither mulch nor mulch x soil conditioner interaction had a significant effect on LWP (Table 2). However, the addition of soil conditioner (New_SC40) significantly improved seedling water status compared to SC-. In contrast, we observed that SC dosage (sub-experiment 2) and SC formulation (sub-experiment 3) had no significant effect on LWP.

SPAD values were not affected by the different mulches. However, the use of New_SC40 resulted in a higher SPAD than SC-. Mulch x soil conditioner interaction was also significant. Exploring this interaction further we found that, in the presence of soil conditioner, all mulches enhanced the SPAD value compared to the Control, while in the absence of soil conditioner no mulch had any effect on SPAD.

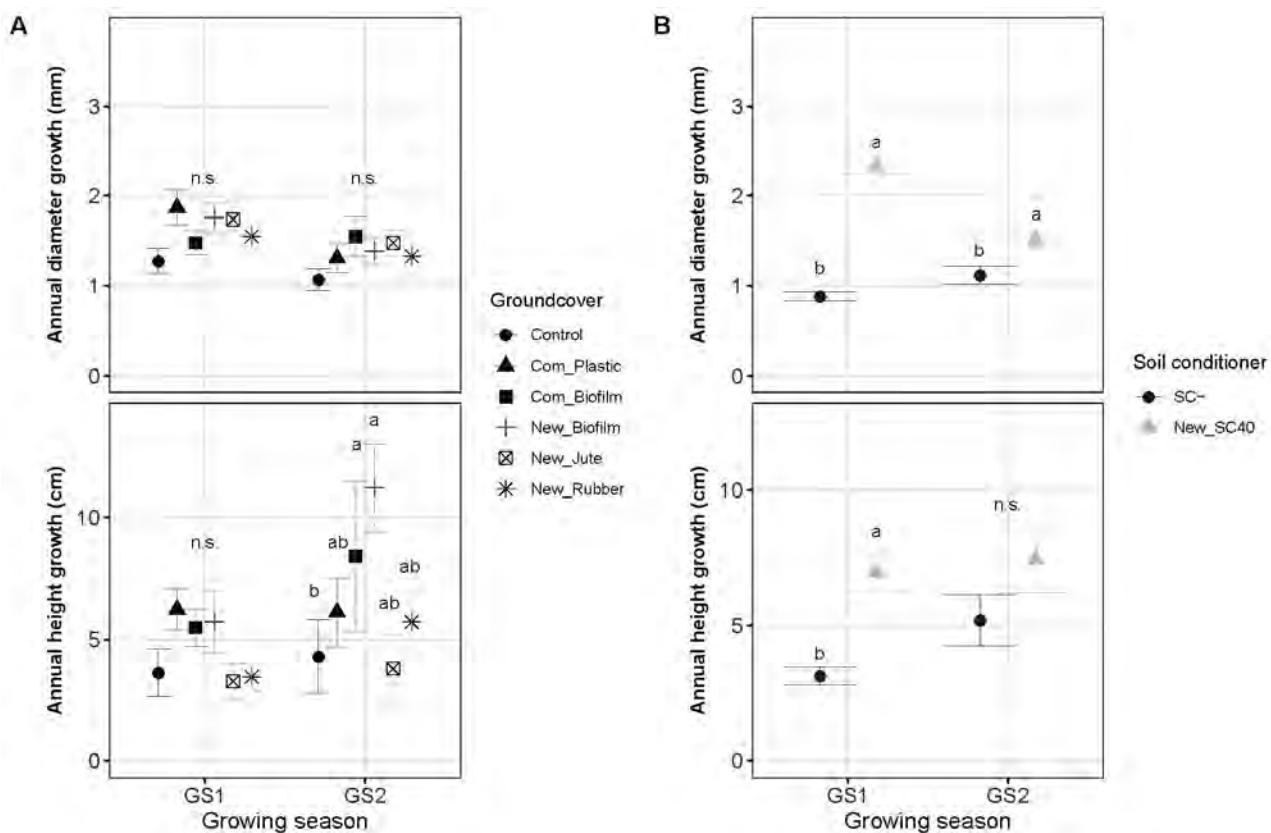


Figure 1. Annual diameter and height growth during the first two growing seasons (GS1-GS2) for sub-experiment 1. A: mulch treatments; B: presence of soil conditioner. For each variable and year, significant differences ($p < 0.05$) between treatments are indicated by different letters (Duncan test grouping), while “n.s.” indicates not significant. Whiskers indicate standard error of the mean.

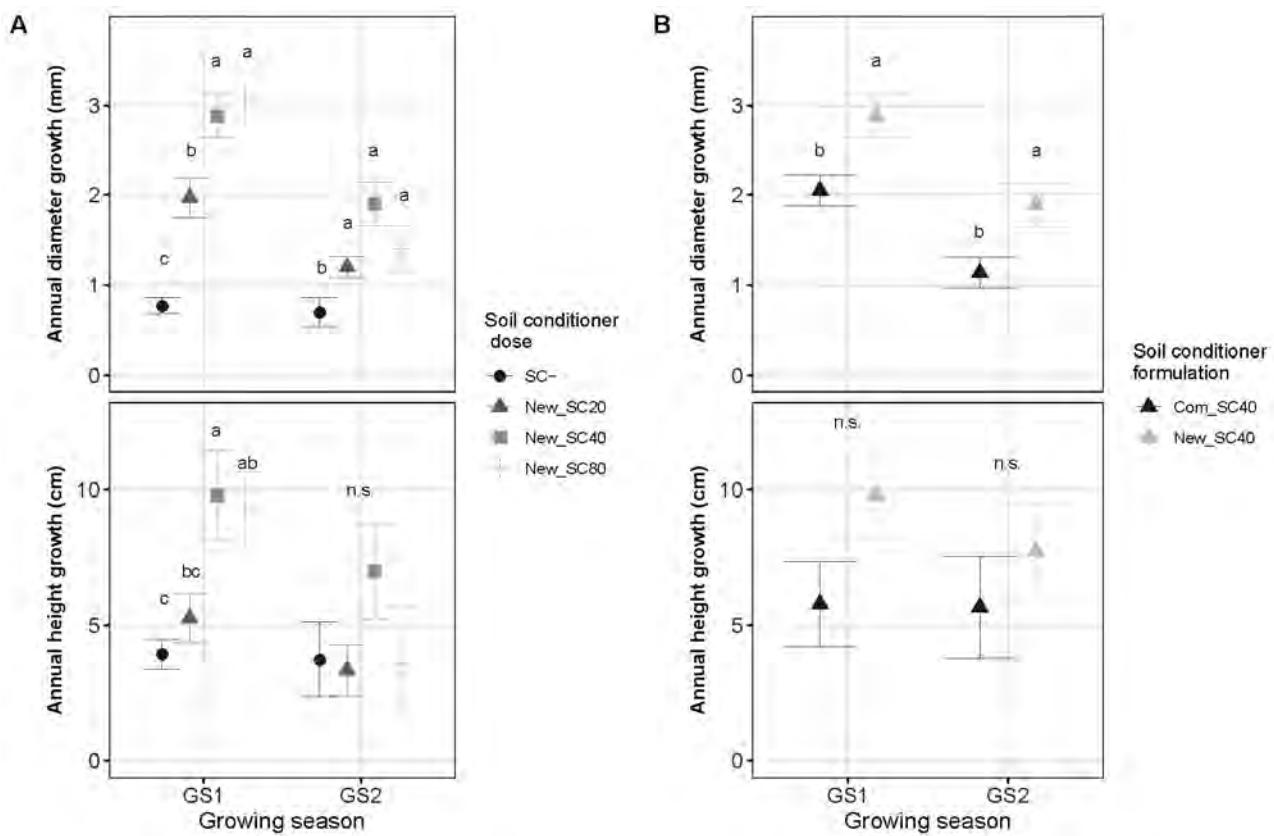


Figure 2. Annual diameter and height growth during the first two growing seasons (GS1-GS2) for (A) sub-experiment 2, and (B) sub-experiment 3. For each variable and year, significant differences ($p < 0.05$) between treatments are indicated by different letters (Duncan test grouping), while “n.s.” indicates not significant. Whiskers indicate standard error of the mean.

Increasing the dose of New_SC (sub-experiment 2) to 40 or 80 g/seedling also led to enhanced SPAD compared to SC-. In sub-experiment 3 the new soil conditioner also resulted in significantly higher SPAD than the commercial one.

Table 3 shows the LWP and SPAD values of each treatment.

Mulch durability

The different mulches showed contrasting levels of damage after three growing seasons (Figure 3). The models with the highest integrity were New_Rubber (97% of units intact or with only slight damage) and Com_Plastic (91%). However, the biodegradable mulches had 20%, 8% and 14% (Com_Biofilm, New_Biofilm and New_Jute respectively) of the units severely damaged, while about half of the units were intact.

Discussion

Our study showed the importance that planting techniques can have on improving the early

performance of broadleaf seedlings in south European mountain afforestation. The hypotheses of sub-experiment 1 were only partially corroborated: the addition of soil conditioner had a positive effect on all seedling performance parameters, but mulching and the interaction between mulch and soil conditioner (which was additive rather than synergistic) led to benefits below our expectations. In sub-experiment 2, the hypothesis was corroborated because the performance of the soil conditioner was better when the dose was increased from 20 to 40 g/seedling; however, there was a saturating effect at the 80 g/seedling dosage. Finally, the hypothesis of sub-experiment 3 was only partially corroborated, as we found similar height growth and seedling water statuses for the new soil conditioner and the commercial formulation, as initially foreseen; however, the new soil conditioner resulted in unexpected gains in diameter growth and seedling nutrient status.

Nevertheless, our results should be verified with further experiments involving a wider variety of tree species, a longer time span, and whenever possible a thorough study of the changes in the soil water

Table 3. Midday Leaf Water Potential (LWP) and SPAD of the measurements taken in the summers of 2014 and 2015. Values are expressed as mean \pm standard error. Significant differences ($p < 0.05$) between treatments are indicated by different letters, according to Duncan's post-hoc test grouping.

| | LWP (bar) | SPAD |
|-------------------------------------------------|-------------------|-------------------|
| Sub-experiment 1 (Mulch and soil conditioner) | | |
| Control | -22.0 \pm 0.3 a | 29.1 \pm 0.7 a |
| Com_Plastic | -21.8 \pm 0.5 a | 30.5 \pm 0.7 a |
| Com_Biofilm | -21.2 \pm 0.4 a | 31.1 \pm 0.7 a |
| New_Biofilm | -22.2 \pm 0.4 a | 29.6 \pm 0.7 a |
| New_Jute | -21.5 \pm 0.5 a | 31.1 \pm 0.7 a |
| New_Rubber | -21.0 \pm 0.4 a | 30.7 \pm 0.7 a |
| SC- | -22.1 \pm 0.3 b | 29.8 \pm 0.4 b |
| New_SC40 | -21.2 \pm 0.3 a | 30.9 \pm 0.4 a |
| Sub-experiment 2 (soil conditioner dose) | | |
| SC- | -21.9 \pm 0.7 a | 28.8 \pm 0.9 b |
| New_SC20 | -22.0 \pm 0.5 a | 30.7 \pm 0.9 ab |
| New_SC40 | -21.7 \pm 0.6 a | 32.3 \pm 0.8 a |
| New_SC80 | -21.8 \pm 0.5 a | 33.1 \pm 1.2 a |
| Sub-experiment 3 (soil conditioner formulation) | | |
| Com_SC40 | -20.9 \pm 0.5 a | 28.9 \pm 0.8 b |
| New_SC40 | -21.7 \pm 0.6 a | 32.3 \pm 0.8 a |

status throughout the seasonal cycle, and how it is affected by the presence of the mulches and/or soil conditioners. Our results may also have been affected by the exceptionally wet summer (50% higher than the reference value) during the first growing season, which could have contributed to the very high survival rate (99%). If the first summer had been drier, the effect of the soil conditioners and especially the mulches may have been more evident because the seedlings would have been exposed to a more intense water stress that would have been alleviated by the two planting techniques.

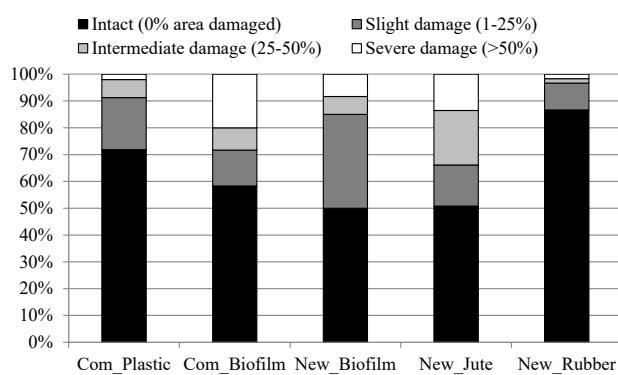


Figure 3. Mulch durability: proportion of the different mulch models in each level of physical damage, after 30 months in the field.

Overall mulch performance

In general, mulching had no significant effect on seedling performance with the only exception of New_Biofilm, which resulted in larger height growth compared to the Control in the second growing season. Although the other variables did not result in significant differences between mulches, the Control was the treatment with the lowest diameter growth and SPAD, and the second lowest height growth and LWP. This seems to imply that mulching has a slightly positive effect. Based on over 110 reports, Van Sambeek (2010) analysed the relative productivity response of different weeding techniques on various tree species, finding a mulch:control response ratio for *Fraxinus* spp. of 222, meaning that mulched seedlings yielded on average 122% more than those without mulch. In our study, however, the mean mulch:control response ratio was 132 for diameter growth and 148 for height growth. Dostálek *et al.* (2007) also found a negligible height growth response in *Fraxinus excelsior* subject to 40 cm wide textile mulching compared to the control, after 5 growing seasons. However, the same authors found a positive effect of fresh bark mulch with similar dimensions as it increased (2-fold) the overall height growth rate compared to the control. The mulches tested here were similar to those evaluated in a previous study (Coello *et al.*, 2018), in which we found no significant

differences between them with regard to seedling water status. In contrast, Paris *et al.* (1998) found that 2 m wide plastic mulching had a positive effect on walnut LWP. However, similarly to our study, Cregg *et al.* (2009) and Coello *et al.* (2017) observed that mulching had no effect on seedling early nutrient status. These results question the interest of mulching in the study conditions. That the mulch performance was below the initial expectations could be due to three factors:

(i) the competitiveness of spontaneous vegetation during the first two growing seasons was poor because (a) the soil preparation left 40 x 40 cm of bare soil prior to planting, and (b) the site quality was low due to low temperatures and a stony, coarse-textured soil. Indeed, most of the unmulched planting pits did not show relevant proliferation of extant vegetation, and therefore the expected gains induced by the weeding effect of mulching could have been masked.

(ii) the mulches in our study were rather small (40 x 40 cm) compared to most previous works. Previous studies with small mulches (60 x 60 cm or less) suggest that there are lower gains in seedling performance (Navarro *et al.*, 2005; Dostálek *et al.*, 2007; Valdecantos *et al.*, 2009, 2014; Coello *et al.*, 2018) compared to the more evident benefits of mulches sized 80 x 80 cm or larger (Jiménez *et al.*, 2014, 2016; Vitone *et al.*, 2016; Coello *et al.*, 2017). Davies (1988) found that the mulches with sides sized 120 cm or larger were more effective than those with sides sized 60 cm or less.

(iii) the summer rainfall during the first growing season was 50% higher than the reference value, which may have masked the positive effect of mulching on soil water retention (Barajas-Guzmán & Barradas 2011, McConkey *et al.*, 2012).

Mulch models

There were only minor differences between the mulch models, in line with Maggard *et al.* (2012) and Coello *et al.* (2017, 2018). Only New_Rubber resulted in a much lower survival rate (82%) than the other mulches (95% in average). Overall, and despite the general lack of significant differences between them, the best performance could be attributed to New_Biofilm, which ranked first in overall seedling growth, and to Com_Biofilm, which ranked second in both LWP and SPAD. The sound performance of these models, together with their degradability and renewable origin, make them, along with New_Jute, suitable alternatives to plastic mulching with added benefits from the technical (do not need to be removed) and environmental points of view.

The durability of the mulches was assessed after 30 months, a time span long enough to provide an initial

idea of their service life. The plastic mulch in the study performed in line with the literature: Haywood (2000) and Coello *et al.* (2017) found respectively 70% and 66% of plastic mulches with 20% or less damaged area after five years, similarly in our case 91% of units had only 20% of damage or less. The biodegradable mulches also performed in line with previous studies: a biofilm similar to New_Biofilm tested by Coello *et al.* (2017) showed 33% of units severely damaged after 40 months of study, while in our case the values ranged between 8% (New_Biofilm) and 20% (Com_Biofilm). We can therefore initially conclude that the biodegradable mulches are expected to meet the desirable service life of 4-5 years during which more than half of the units should remain intact or be only slightly damaged (Coello & Piqué, 2016).

Overall performance of soil conditioners

The use of SCwSAPs was positive for early seedling performance, with an overall improvement in all assessed parameters, in line with a previous study with the same products evaluated at an afforestation site with *Pinus halepensis* in a coarse-textured soil in semiarid conditions (Coello *et al.*, 2018). However, these results contrast with most previous afforestation studies using SCwSAPs, which found negligible or even negative effects when the SCwSAPs were applied inadequately (1.5 years after planting – Bulíř, 2006; superficial application – Hueso-González *et al.*, 2016), in a low or inaccurate dosage (15 g/seedling – Navarro *et al.*, 2005; unknown dose – Frigola & Nadal, 2013) or in fine-textured, clayish soils (Bulíř, 2005; Navarro *et al.*, 2005; Del Campo *et al.*, 2011; Frigola & Nadal, 2013).

After two growing seasons the average survival for seedlings including any type or dose of soil conditioner (New_SC20, New_SC40, New_SC80 and Com_SC40) was 95%, slightly higher than the 90% of SC-seedlings. Hüttermann *et al.* (2009) also reported a positive effect of soil conditioners on the survivorship of various species and Chirino *et al.* (2011) found a positive effect for *Quercus ilex* L.

With regard to annual diameter and height growth, New_SC40 seedlings grew 92% and 72% more than those of the control treatment, similarly to our previous study (Coello *et al.*, 2018). The growth gains induced by pure SAP (not including fertilizers and other components present in the SCwSAPs tested) in previous afforestation studies with broadleaved species are much less evident than in the present study (Rowe *et al.*, 2005; with *Alnus cordata* (Loisel.) Dub., *Alnus glutinosa* (L.) Gaertn and *Salix x reichardtii*) or even negligible (Clemente *et al.*, 2004 with *Ceratonia*

siliqua L., *Olea europaea* L. and *Pistacia lentiscus* L.; Chirino *et al.*, 2011 with *Quercus suber* L.).

The positive effect of SCwSAP or pure SAP on seedling water status has been reported in nursery conditions for other broadleaved species: *Citrus* sp (Arbona *et al.*, 2005); *Populus euphratica* Oliv. (Luo *et al.*, 2009); *Quercus suber* (Chirino *et al.*, 2011) and *Fagus sylvatica* L. (Beniwal *et al.*, 2011; Jammicka *et al.*, 2013). The improved water status induced by the SCwSAP suggests that the seedlings use the water stored by the polymer (Del Campo *et al.*, 2011; Jammicka *et al.*, 2013). The improvement in nutrient status induced by New_SC40 was also described by Machado *et al.* (2016), who reported an increase in the level of macro and micronutrients in soils when a SCwSAP was used. In contrast, the use of pure SAP by Clemente *et al.* (2004) did not increase seedling chlorophyll or nitrogen content. This suggests that some of the ingredients of New_SC other than SAP (probably the fertilizers, humic acids, and/or the root growth precursors) help improve tree nutrient status, which is a relevant advantage in soils with a poor nutrient retention capacity, i.e. coarse textured and/or stony soils.

The apparent reduction over time in the soil conditioner effect, which was detected in this study in the second growing season, was also reported by Chirino *et al.* (2011), Oliveira *et al.* (2011) and Coello *et al.* (2018), and could be because (i) the nutrients added via the soil conditioner are progressively exhausted, and/or (ii) the seedling develops new roots out of the planting pit, thus accessing unconditioned soil volume.

Soil conditioner dosage and formulation

Increasing soil conditioner dosage (sub-experiment 2) was generally positive, in line with Al-Humaid & Moftah (2007) and Hüttermann *et al.* (2009). The most cost-effective dose was 40 g/seedling, coinciding with the manufacturer's recommendation for 30 x 30 x 30 cm soil mixing volume. This finding is also in line with our previous study (Coello *et al.*, 2018), in which we found that the 20 g/seedling dose led to better results than the control less often than the 40 g/seedling dose, which provided outcomes that were similar to the highest dose (80 g/seedling) for all variables. Future cost-effectiveness studies considering various dosages, as well as the application of this product at an operational scale, could be used to fine tune the viability and the most recommendable dosage for different site conditions.

In sub-experiment 3 New_SC40 led to better results than Com_SC40 for diameter growth (both growing seasons) and SPAD. These findings contrast with our previous study in semiarid conditions (Coello *et al.*,

2018), in which we generally found similar results for both products. This suggests that the ingredients contained in the new formulation and not in the commercial one (humic acids and the type of root growth precursors and polymers) may be particularly beneficial in cold, montane conditions or for nutrient-demanding species such as *Fraxinus excelsior*. Another strength of the new formulation is that it is expected to have higher social acceptability than most commercially available SAPs or SCwSAPs that contain polyacrylamide (DRI, 2008), like the commercial model in our study. Future experiments with these products will make it possible to ascertain the site conditions and species for which each formulation is particularly efficient.

Interactions between mulch and soil conditioner

As mulching had predominantly no or only minor effects, applying this technique combined with the soil conditioner seems to be of little interest in these conditions. The interaction between the two techniques was only significant in the case of SPAD, where the soil conditioner combined with any mulch type resulted in better seedling nutrient status than when applied alone (unmulched seedlings), while this did not occur in the absence of soil conditioner. This suggests that, for this particular variable (SPAD), the positive effects of mulching may be enhanced when fertilizers are added to the plantation pit, in line with Vincent & Davies (2003).

Implications for management

The soil conditioner with water super-absorbent polymers in a synergistic mixture with other ingredients (fertilizers, humic acids and root growth precursors) is a cheap and easily applied technique that does not require maintenance, and which had a positive effect on all seedling performance indicators in our study. The tested polyacrylamide-free prototype increased both seedling water status, probably due to the polymer; and nutrient status, probably due to the fertilizers and humic acids. This technique ultimately increased seedling survival and growth. The most cost-effective soil conditioner dose was the one recommended by the manufacturer, 40 g/seedling, which often led to better results than the lower dose (20 g/seedling) but never worse than the higher one (80 g/seedling). The new prototype tested seems a suitable alternative to the commercially available version, with technical and social advantages: higher growth rates and better seedling nutrient status, while also being polyacrylamide-free.

On the other hand, the use of small mulches (40 x 40 cm) had a slightly positive effect compared to untreated

seedlings, which was usually not statistically significant. Therefore, this technique does not seem a first priority option for enhancing the early establishment of seedlings at poor-quality, montane afforestation sites that have low competitiveness of extant vegetation. Among the different mulch models, the biodegradable ones (prototypes based on biofilm and woven jute, as well as the commercial biofilm) performed similarly to the plastic mulch, and therefore show added value from the technical (do not require removal) and social (come from renewable sources) perspectives.

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