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Efectos del biochar sobre el suelo, las características de la raíz y la producción vegetal

Manuel Olmo Prieto

Directores: Rafael Villar Montero y José Antonio Alburquerque Méndez

Córdoba, 11 de Enero de 2016

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TÍTULO DE LA TESIS

Efectos del biochar sobre el suelo, las características de la raíz y la producción vegetal

DOCTORANDO

Manuel Olmo Prieto

INFORME RAZONADO DE LOS DIRECTORES DE LA TESIS

El trabajo presentado por Manuel Olmo Prieto titulado "*Efectos del biochar sobre el suelo, las características de la raíz y la producción vegetal*" constituye la memoria de su tesis doctoral.

Esta tesis doctoral consta de 6 capítulos: una introducción general, 4 capítulos experimentales y una discusión general.

El tema principal de la tesis se centra en los efectos del biocarbón (biochar) sobre las características del suelo y cómo estos cambios influyen sobre el crecimiento de las plantas y la producción vegetal. Los resultados de la tesis son muy interesantes y novedosos, ya que permiten conocer de una forma más profunda cuáles son algunos de los efectos del biochar sobre los rasgos funcionales de las plantas. Los resultados obtenidos son generalizables ya que comprenden un gran número de especies estudiadas, así como bajo distintas aproximaciones (experimentos en cámara, invernadero y campo). Dos capítulos están publicados en revistas internacionales dentro del primer cuartil y los otros dos capítulos experimentales están actualmente en revisión en otras revistas internacionales. Además, gran parte de estos resultados se han divulgado en noticias que han aparecido en periódicos, páginas web y en la radio.

Por todo ello, se autoriza la presentación de la tesis doctoral.

Córdoba, 11 de Enero de 2016

Fdo.: Rafael Villar Montero Fdo.: José Antonio Alburquerque Méndez

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<u>Publicaciones en revistas científicas (Capítulo de la tesis doctoral)</u>

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<u>Manuscritos enviados a revistas científicas (Capítulo de la tesis doctoral)</u>

Olmo M, Villar R (2015) Biochar affects fruit production in eight agronomic species through changes in root traits. Manuscrito enviado a Agriculture Ecosystems & Environment. (**Capítulo 3)**

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Otros trabajos en los que ha colaborado

Olmo M, Lopez-Iglesias B, Villar R (2014b) Drought changes the structure and elemental composition of very fine roots in seedlings of ten woody tree species. Implications for a drier climate. Plant and Soil 384:113–129

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de la Riva EG, Pérez-Ramos IM, Tosco A, Navarro-Fernández CM, **Olmo M**, Marañón T, Villar R (2015). Disentangling the relative importance of species occurrence, abundance and intraspecific variability in community assembly: a trait-based approach at the whole-plant level in Mediterranean forests. Oikos (*en prensa*). DOI: 10.1111/oik.01875

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Otros méritos académicos

Premio al mejor trabajo fin de máster en producción, protección y mejora vegetal (Universidad de Córdoba, 2013). Efecto del biocarbón sobre el crecimiento y producción de un cultivo de trigo en condiciones de campo.

El presente trabajo se ha realizado en el marco del Proyecto de Investigación IPT-440000-2010-8 titulado "***Proyecto Biocar: Estudio del biocarbón como sumidero de carbono***" financiado por el Ministerio de Ciencia e Innovación (Programa Nacional de Cooperación Público-Privada, Subprograma INNPACTO) y fondos FEDER ''Fondo Europeo de Desarrollo Regional, una manera de hacer Europa".

Gracias por vuestra paciencia y comprensión, me habéis dado vuestro tiempo para que yo cumpliera con el mío.

A mis padres y a mi mujer

"Biochar, una de las nuevas estrategias más excitantes para restaurar el carbono de los suelos pobres y secuestrar grandes cantidades de CO_2 durante miles de años"

Al Gore, Vicepresidente de los Estados Unidos y galardonado con el Premio Nobel de la Paz (2007)

Índice

Resumen

La agricultura debe hacer frente en la actualidad a una serie de desafíos como son el cambio climático, la degradación y pérdida de fertilidad de los suelos, y la alta demanda de alimentos. El sector agrícola es muy relevante en este escenario, ya que, por una parte, la actividad agrícola contribuye a la emisión de gases de efecto invernadero y por otra, se ve afectada negativamente por las consecuencias derivadas del cambio climático (incremento de temperatura, aumento de la aridez, etc.). Hasta ahora se ha conseguido aumentar el rendimiento de los cultivos principalmente mediante prácticas poco sostenibles a largo plazo, tales como el manejo abusivo del suelo y el uso excesivo de fertilizantes. Todo ello a expensas de la reducción de la calidad del suelo y del aumento de la contaminación del mismo. Esto hace necesario implementar en el sector agrícola estrategias sostenibles que aumenten el rendimiento de los cultivos sin aumentar el impacto sobre el área cultivada y que reduzcan a su vez las emisiones de gases de efecto invernadero.

El biochar es un material rico en C que se obtiene de la descomposición termo-química de residuos orgánicos a temperaturas que generalmente oscilan entre 300 y 700 ˚C y en ausencia de oxígeno (pirólisis). El proceso de pirólisis estabiliza el C existente en la materia orgánica en una forma más resistente a la descomposición química y biológica, por lo que al ser incorporado al suelo no se degrada y el C no es emitido a la atmósfera como ocurre con la descomposición de materia orgánica sin pirolizar. Las características del biochar le confieren la capacidad potencial de mejorar las propiedades físico-químicas del suelo y aumentar la productividad de los cultivos, contribuyendo además al secuestro de C, lo que convierte al biochar en una herramienta para luchar contra el cambio climático.

Los efectos del biochar sobre las propiedades del suelo pueden varían en función de las características del biochar, que a su vez dependen de las propiedades del material del que se obtiene y de las condiciones de pirólisis. Esto explica que las respuestas de los cultivos a la adición de biochar sean muy variables. La mayoría de los estudios han analizado los efectos del biochar sobre la producción agrícola, sin profundizar en los mecanismos de las plantas que explican estas respuestas. Esta tesis doctoral tiene como objetivo profundizar en el estudio de estos mecanismos, evaluando las respuestas de los rasgos funcionales más importantes de la planta a los cambios inducidos por el biochar.

Para ello, se han realizado experimentos tanto en condiciones controladas (cámara de cultivo e invernadero) como en campo, usando diferentes biochars (procedentes de poda de olivo y paja de trigo), dosis de aplicación y especies agronómicas: algodón (*Gossypium herbaceum* L.), berenjena (*Solanum melongena* L.), colza (*Brassica napus* L.), garbanzo (*Cicer arietinum* L.), maíz (*Zea mays* L.), pimiento (*Capsicum annuum* L.), soja (*Glycine max* L.), tomate (*Solanum lycopersicum* L.) y trigo (*Triticum durum* L.).

Nuestros resultados indicaron que las características de los biochars variaron dependiendo del material de partida, pero en general presentaron un pH alcalino, alto contenido de C y bajo contenido de nutrientes. De manera general, la adición de biochar redujo la densidad aparente y la compactación del suelo y aumentó su capacidad de retención hídrica. La adición de biochar ejerció una gran influencia sobre la disponibilidad de nutrientes en el suelo, aumentando la disponibilidad de P, K, Ca, Mg y Cu, y reduciendo la de N.

Los cambios inducidos por los biochars sobre las propiedades de los suelos afectaron a la morfología de la raíz. En general, la adición de biochar aumentó la longitud específica de la raíz y redujo su diámetro y densidad tisular. La adición de biochar ejerció un impacto positivo sobre el estado hídrico de las plantas y aumentó la producción de biomasa de la parte aérea, lo que promovió el crecimiento y la producción vegetal. Tanto en los experimentos de adición del biochar en invernadero como en los de campo encontramos una relación positiva y significativa entre la longitud específica de la raíz y la producción vegetal, lo que sugiere que el aumento de la proliferación de raíz fina tras la adición de biochar aumentó el rendimiento del cultivo. Este hecho fue relacionado fundamentalmente con un mayor acceso a los recursos disponibles, favorecido tanto por un mayor volumen de exploración como una mayor interacción partículas de biochar-raíz. Otro hecho a destacar es que la adición de biochar junto con una fertilización siempre mejoró los rendimientos obtenidos respecto a la sola aplicación de la fertilización, lo que sugiere que su adición mejoró la eficacia del fertilizante añadido.

En la práctica agrícola es de suma importancia asegurar una correcta incorporación del biochar en el suelo. Debido a que el tamaño de partícula del biochar es variable, su mezcla con el suelo puede ser poco homogénea. Además, los movimientos del suelo debidos a su manejo (arado y siembra) pueden acrecentar este problema. La mayoría de estudios no consideran estos cambios que afectan de forma considerable a la distribución del contenido de biochar en el suelo, lo que puede dar lugar a resultados confusos y por tanto no concluyentes. Los resultados de nuestro estudio mostraron que se puede estimar el contenido de biochar en el suelo mediante una técnica sencilla que consiste en una categorización visual en el campo basada en el color oscuro que el biochar confiere al suelo. Esta categorización visual correlacionó positivamente tanto con el contenido de biochar del suelo como con la producción de trigo y, por tanto, puede emplearse como una herramienta útil para comprobar la incorporación homogénea del biochar en el suelo.

En resumen, los resultados sugieren que el biochar puede ser usado para mejorar la calidad del suelo, aumentar la eficiencia de los fertilizantes y la producción de un amplio rango de especies agronómicas. En este sentido, cabe destacar como aspectos clave para asegurar la eficacia del biochar: *i*) adecuar las características del biochar a las propiedades del suelo y condiciones de cultivo; y *ii*) determinar qué factores condicionan una mayor disponibilidad de nutrientes en el sistema suelo-biochar-planta, especialmente aquellos nutrientes retenidos en el biochar.

Summary

Agriculture has currently to face challenges such as climate change, soil degradation and loss of fertility and high demand for food production. The agricultural sector is very important under the above scenario, since, agricultural activity contributes to greenhouse gas emissions and, it is adversely affected by the consequences of climate change (temperature increase, aridity, etc.). So far the crop yield increase has been mainly obtained by unsustainable practices in the long-term such as abusive land management and overuse of fertilizers, at the expense of reduced soil quality and increased pollution. Therefore, it is necessary to implement sustainable agriculture strategies to increase crop yields without increasing the impact on cultivated area and reduce greenhouse gas emissions.

Biochar is a carbon-rich material obtained from the thermochemical decomposition of organic wastes at temperatures between 300 and 700 ° C under oxygen limited conditions (pyrolysis). The pyrolysis process stabilizes the C existing in the organic matter in a more resistant to chemical or biological decomposition, so that when incorporated into the soil is kept stable for longer and is not emitted to the atmosphere as would occur with the biomass decomposition. Biochar characteristics give it the capacity to improve the physic-chemical properties of soil and increase crop yield, while contribute to C sequestration, which becomes a tool to fight against climate change.

The effects of biochar on soil properties depend on the characteristics of biochar, which in turn depend on the characteristics of feedstock and pyrolysis conditions. This explains the high variability in crop responses obtained after biochar addition. Most biochar studies have examined its effects on crop yield, without deepening in the plant mechanisms that explain these responses. The main objective of this thesis is to deepen in these plant mechanisms, evaluating the responses of key functional plant traits to biochar-induced changes. For this purpose, experiments both under controlled (growth chamber and greenhouse) and field conditions were carried out, using different biochar types (obtained from olive-tree pruning and wheat straw), application rates and agronomic species: cotton (*Gossypium herbaceum* L.), eggplant (*Solanum melongena* L.), rapessed (*Brassica napus* L.), chickpea (*Cicer arietinum* L.), corn (*Zea mays* L.), pepper (*Capsicum annuum* L.), soybean (*Glycine max* L.), tomato (*Solanum lycopersicum* L.) and wheat (*Triticum durum* L.).

Our results indicate that biochar characteristics differ depending on the feedstock used, but generally, biochars showed an alkaline pH, high C content and low nutrient content. In general, biochar addition reduced soil bulk density and compaction and increased soil water retention capacity. The biochar addition exerted a great influence on soil nutrient availability, increasing P, K, Ca, Mg and Cu, and decreasing N.

The results showed that changes in soil properties induced by biochar addition affected root morphology. In general, biochar increased the specific root length and reduced root diameter and root tissue density. Biochar addition increased plant water status and biomass production of the aerial part, which promoted plant growth and production. In both greenhouse and field experiments, we found a positive relationship between the biochar effect on the specific root length and the biochar effect on crop production, suggesting that the increased proliferation of fine roots following the addition of biochar enhanced crop yield. This was mainly related to a greater access to available resources, favored by higher soil exploration and biochar particles-root interactions. Another fact to note is that the addition of biochar combined with a fertilization always improved crop yield compared to the sole application of the fertilization, suggesting that its addition improved the efficiency of the fertilizer added.

In the agricultural practice it is important to ensure a proper incorporation of biochar into the soil. Since the particle size of biochar varies considerably, its mixing with the soil can lead to a non-homogeneous spatial distribution. In addition, soil movements associated to standard agronomic practices such as tillage and sowing may increase this problem. Most studies do not consider these changes, which can lead to confusing and therefore inconclusive results. Our study showed that we can estimate the content of biochar in the soil using a simple technique of visual categorization based on the dark color associated to biochar. This visual categorization was positively correlated with both biochar content and wheat yield, and therefore it can be used as a useful tool to assess the homogeneous distribution of biochar into soil.

In summary, the results suggest that biochar can be used to improve soil quality, increase fertilizer efficiency and crop production of a wide range of agronomic species. In this regard it is noteworthy as key aspects to ensure the effectiveness of biochar: *i*) to adjust the characteristics of biochar to soil properties and crop conditions; and *ii*) to determine what factors are involved in a greater availability of nutrients in the soil-biochar-plant system, especially those nutrients retained in biochar.

Capítulo 1. Introducción general

Actualmente la concentración de gases de efecto invernadero en la atmósfera (principalmente CO_2, CH_4 y óxidos de nitrógeno) sigue aumentando, debido principalmente al uso de combustibles fósiles, lo que acelera el calentamiento global y está alterando el clima a escala global (IPCC 2014). La mayoría de estas emisiones son de origen antrópico (Stern et al. 2014), concretamente la agricultura y actividades asociadas generan al año entre el 10 y el 12 % de las emisiones antropogénicas de CO_2, junto con la mayoría de las emisiones de óxidos de nitrógeno procedentes de la actividad microbiana del suelo (nitrificación y desnitrificación) y del uso excesivo de fertilizantes nitrogenados (Davidson et al. 2009; Smith et al. 2014). Es por tanto necesario aportar mejoras para promover una agricultura sostenible a largo plazo. La implementación de técnicas agronómicas más sostenibles es fundamental para evitar y reducir fenómenos de contaminación y emisiones gaseosas, mitigar el cambio climático y mejorar la calidad del suelo y el rendimiento de los cultivos.

El uso de biochar (biocarbón en español) como enmienda de suelo puede ser una solución viable para la mejora de la calidad del suelo y la captura de C. El biochar (biocarbón, en español) es un material rico en C, obtenido a partir de residuos orgánicos (restos vegetales, residuos forestales, estiércol animal, etc.) mediante la descomposición térmica a temperaturas relativamente bajas (< 700 ˚C) y en una atmósfera ausente o pobre en oxígeno (pirólisis). Este proceso estabiliza el C de la materia orgánica haciéndolo más resistente a la descomposición química y biológica (Lehmann y Joseph 2009, 2015). El biochar se diferencia del carbón vegetal (charcoal, en inglés) en que su finalidad es aplicarlo al suelo para la mejora de sus propiedades y el secuestro de C, mientras que el carbón vegetal se utiliza para ser quemado y obtener energía (Lehmann y Joseph 2009, 2015).

El biochar puede jugar un papel importante en la agricultura, ya que tiene el potencial de reducir las emisiones de CO_2, actuando como sumidero de C (Fig. 1). Además, su producción puede conllevar el aprovechamiento energético de los bio-aceites y gases originados durante el proceso de pirólisis, reduciendo así la utilización de combustibles fósiles (Lehmann y Joseph 2009, 2015). Otro papel importante del biochar puede ser mejorar la fertilidad del suelo, reduciendo la necesidad de fertilizantes y mitigando las emisiones de óxidos de nitrógeno, además de constituir una herramienta útil para la gestión de residuos orgánicos (Fig. 1) (Jeffery et al. 2015).

Figura 1. Principales objetivos de la producción de biochar (modificado a partir de Lehmann y Joseph 2009).

Hoy en día, un aspecto clave del que depende la implantación del proceso de producción de biochar y su uso como enmienda de suelos es evaluar su rentabilidad económica. Existen estudios tanto a favor (Joseph 2009; Galinato et al. 2011) como en contra (McCarl et al. 2009; Liu et al. 2013), lo que demuestra su complejidad debido al gran número de variables que intervienen. Sin embargo, la mayoría de estos estudios coinciden en aquellos aspectos que aportarían al biochar mayor valor económico: *i)* tratar de dilucidar los aspectos beneficiosos del biochar; *ii)* la obtención de biochar a partir de residuos y así reducir los costes del proceso; *iii)* la introducción en los mercados del biochar como una herramienta consolidada para mitigar el cambio climático; y *iv)* la puesta en marcha de políticas que proporcionen ingresos económicos adicionales.

Estudio y origen del biochar

El concepto de biochar es relativamente reciente y el interés por su aplicación a suelos agrícolas se debe principalmente al descubrimiento de sustancias de naturaleza similar al biochar en tierras oscuras de la Amazonia, conocidas localmente como *Terra preta do indio*. Estos suelos son ricos en C orgánico y muy fértiles, lo que representa una anomalía respecto a los suelos de la selva Amazónica que suelen ser muy pobres en nutrientes (Fig. 2; Lehmann y Joseph 2009, 2015).

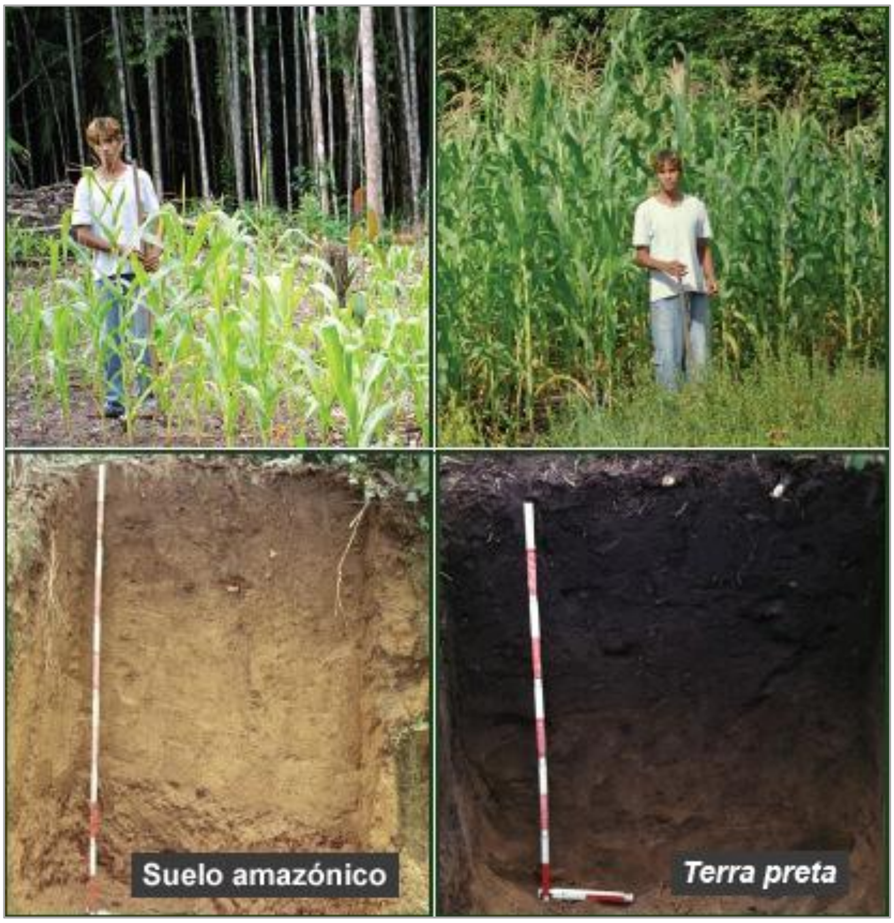

Figura 2. A la izquierda, un suelo amazónico (oxisol) pobre en nutrientes; a la derecha un oxisol transformado en *Terra preta* fértil (fuente: IBI, *Iniciativa Internacional por el Biochar*).

Actualmente se considera que la *Terra preta* amazónica es el producto de las sociedades humanas que habitaron la región mucho antes de la llegada de los europeos (800 aC - 500 dC; Lehmann y Joseph 2009, 2015). Existen diversas teorías para explicar su formación antrópica a partir de suelos oxisoles pobres en nutrientes. Una de ellas considera que estos suelos se formaron por la deposición de material pirogénico derivado de la quema de restos vegetales junto con otros materiales no combustibles procedentes de asentamientos humanos (restos de vasijas, esqueletos de pescado, etc.). Otra teoría considera la quema intencionada de bosques y maleza para la obtención de tierras de cultivo (Glaser y Woods 2004). De aquí nació la idea de quemar residuos orgánicos y mezclarlos posteriormente en el suelo para aumentar su fertilidad y la producción vegetal. A finales del siglo XIX se desarrollaron algunas investigaciones que corroboraron la existencia de unos suelos oscuros y muy fértiles en la Amazonia sin precisar su origen (Glaser y Woods 2004). Fue durante la década de los años ochenta cuando se intensificó su investigación (Smith 1980) y en los últimos años ha experimentado su mayor auge en la literatura científica.

Producción de biochar: El proceso de pirólisis

La pirólisis se define como un proceso termoquímico mediante el cual el material orgánico se descompone, por la acción del calor en una atmósfera deficiente de oxígeno, y se transforma en una mezcla de hidrocarburos, gases combustibles, residuos de carbón y agua (Mohan et al. 2006; Balat et al. 2009). Las transformaciones físicas y químicas que ocurren durante la pirólisis son complejas y dependen en gran medida de la naturaleza de la biomasa inicial y de las condiciones de pirólisis (temperatura, presión y tiempo de residencia del material en el reactor) (Lua et al. 2004).

Los biochars pueden obtenerse a partir de un amplio rango de materiales (residuos agrícolas y forestales, estiércoles, residuos urbanos, lodos de aguas residuales, etc.), cuyas características van a determinar las propiedades físico-químicas del biochar producido (Sohi et al. 2010; Song y Guo 2012). Mukome et al. (2013) analizaron 85 tipos de biochars obtenidos a partir de distintos tipos de materiales, encontrando diferencias significativas entre ellos, así por ejemplo, encontraron que el contenido de nutrientes de los biochars obtenidos a partir de estiércoles fue considerablemente mayor que el de los biochars obtenidos a partir de residuos leñosos. También, las condiciones de pirólisis pueden originar biochars con diferentes características pese a ser obtenidos a partir de un mismo tipo de material (Gaskin et al. 2008; Novak et al. 2009; Brewer et al. 2009). En general, a medida que aumenta la temperatura de pirólisis disminuye el rendimiento de producción de biochar, pero éste se compone por C en estructuras aromáticas más condensadas (más estable a la degradación), además, suelen presentar mayor área superficial y contenido de cenizas, y un pH más alcalino (Glaser et al. 2002; Gaskin et al. 2008; Novak et al. 2009).

Características del biochar

Como hemos mencionado con anterioridad, las características de cada biochar pueden variar considerablemente dependiendo del material de partida y de las condiciones de pirólisis, sin embargo, los biochars comparten una serie de características comunes que se detallan a continuación.

Los biochars poseen un contenido elevado de C recalcitrante, en su mayor parte condensado en anillos aromáticos (Sombroek et al. 2003; Amonette y Joseph 2009), lo que le confiere su elevado potencial de secuestro de C (Lehmann et al. 2006; Xu et al. 2012). También cabe destacar la presencia de nutrientes asociados a su fracción mineral (K, Ca, Mg, P, S, etc.) (Lehmann y Joseph 2009, 2015). La mayoría de los biochars son alcalinos (pH > 7) y dependiendo de la dosis aplicada al suelo, pueden ejercer un efecto de encalado sobre el mismo (Van Zwieten et al. 2010).

En general, los biochars son materiales porosos, poco densos, y caracterizados por una elevada área superficial específica (Lehmann y Joseph 2009, 2015). Esta propiedad determina la reactividad y la capacidad del biochar para retener iones en su superficie (Kookana et al. 2011; Graber et al. 2012). Por su parte, los poros son también responsables de la elevada capacidad de retención de agua del biochar (Brewer et al. 2009; Abel et al. 2013; Ulyett et al. 2014).

En la actualidad, diferentes organizaciones (“International Biochar Initiative”, “European Biochar Foundation”, “British Biochar Foundation”, etc.) han definido y propuesto un conjunto de características con el objetivo de definir la calidad del biochar para su uso en agricultura. Estos parámetros incluyen la distribución del tamaño de partículas del biochar, el pH, el área específica, la porosidad, el contenido de C y nutrientes, así como el contenido de contaminantes (metales pesados, hidrocarburos aromáticos policíclicos, etc.). Muy pocos países poseen una normativa específica para este tipo de materiales, así que estas normas de calidad agronómica constituyen un punto de partida para el desarrollo de una futura legislación que regule la aplicación de biochar al suelo.

Biochar como sumidero de carbono

El suelo actúa como un gran almacén de C, ya que más del 80% del C terrestre se encuentra almacenado en él (Swift 2001). Sin embargo, el potencial del suelo para actuar como sumidero de C es reducido, ya que la captura de éste no es permanente, sino que existe un balance entre la incorporación de materia orgánica al suelo y la salida de C en forma de CO_2 a la atmósfera asociada a procesos tales como descomposición, erosión y lavado (Swift 2001). Por otra parte, la captura del C en el suelo es compensada con la emisión de otros gases que contribuyen al efecto invernadero (Schlesinger 1999). Por lo tanto, para mitigar el cambio climático es necesario reducir el flujo neto de estos gases a la atmósfera, tratando de incidir sobre el ciclo del C.

La adición de biochar al suelo se ha planteado como una opción viable de reducir la concentración de CO_2 atmosférico, favoreciendo la retención de C en el suelo (Lehmann y Joseph 2009, 2015). El potencial del biochar como sumidero de C se debe a su naturaleza recalcitrante, lo que ralentiza la velocidad a la que se degrada en el suelo (Sombroek et al. 2003; Liang et al. 2008) y por tanto la velocidad a la que el C se emite a la atmósfera. El tiempo de residencia media del C del biochar en los suelos es variable pero según estimaciones alcanza los 1000 años, pudiendo llegar hasta los 10000 años (Swift 2001; Zimmerman 2010). En la Fig. 3 se muestra un esquema resumido del impacto del biochar sobre el ciclo del C que abarca desde la producción de biochar a partir de restos vegetales hasta su incorporación al suelo, con la finalidad de secuestrar C.

Amonette et al. (2007) estimaron la reducción de las emisiones de C que se lograrían con la producción y la adición al suelo de biochar. Según este estudio, a nivel terrestre son fijadas por la fotosíntesis 60 gigatoneladas (Gt= 10^9 t) de C atmosférico cada año. Considerando que la pirólisis transforma el 50 % del C del material inicial en biochar y 50 % del C restante en bio-aceites y syngas, que pueden usarse como sustitutos de combustibles fósiles para obtener energía (con una eficiencia media del 62 %); de 1 Gt de C de la biomasa inicial se obtendría 0.5 Gt de C en forma de biochar (secuestrable) y se evitarían la emisión a la atmósfera de 0.3 Gt de C (0.5 Gt C × 0.62) procedentes de los combustibles fósiles. Por tanto, si un 10 % de la biomasa (6 Gt de C) se usase para producir biochar se secuestrarían 3 Gt de C año^{-1} en el suelo y evitaría la emisión 1.8 Gt de C año^{-1} de origen fósil. Esto supondría reducir en 4.8 Gt de C año^{-1} las emisiones a la atmósfera. Considerando que el sector agrícola emite aproximadamente 7.1 Gt año^{-1} (FAO 2013), con el uso de biochar reduciríamos un 68 % de las emisiones de CO_2 procedentes del sector agrícola.

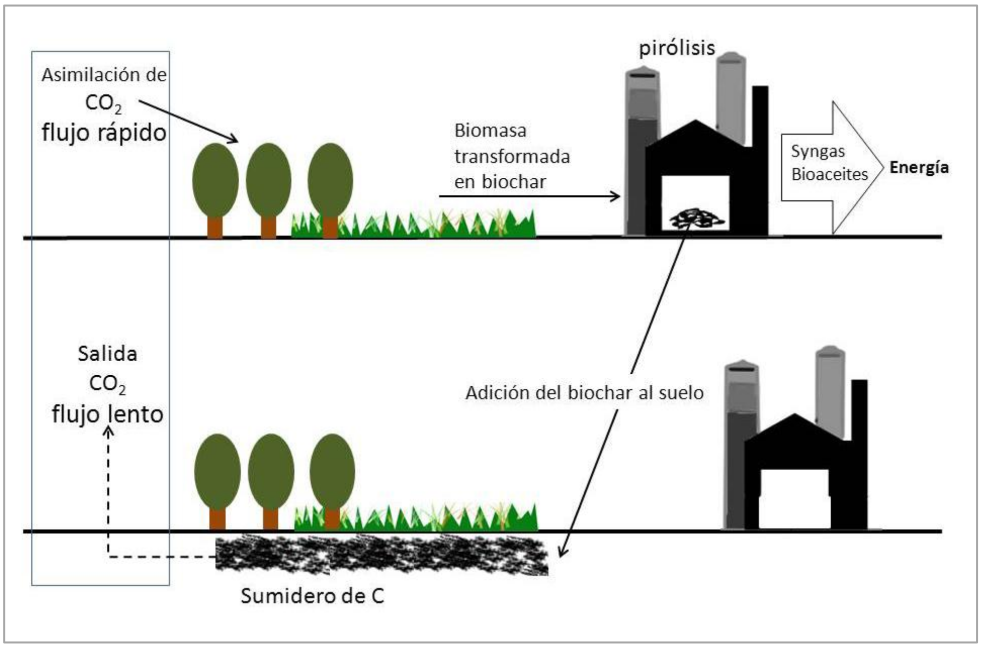

Figura 3. Impacto del biochar sobre el ciclo de C (reducción de las emisiones de CO_2 hacia la atmósfera).

Biochar como enmienda de suelos agrícolas

Las propiedades del biochar lo convierten en un producto interesante como enmienda de suelos. Algunas revisiones bibliográficas han demostrado que el biochar puede mejorar las características del suelo donde se adiciona (Atkinson et al. 2010; Sohi et al. 2010; Lehmann et al. 2011; Ahmad et al. 2014).

La Fig. 4 muestra de modo esquemático los principales efectos observados del biochar sobre las propiedades del suelo. Estos cambios incluyen la reducción de su densidad aparente, el aumento de su capacidad de retención de agua y la mejora de su estructura (Laird et al. 2009; Makoto y Yasuyuki

2010; Baronti et al. 2014). Estas mejoras también incluyen un aumento de la porosidad del suelo que puede mejorar su capacidad de infiltración y su permeabilidad (Abel et al. 2013; Ulyett et al. 2014), contribuyendo positivamente al desarrollo de la raíz y a la respiración microbiana y favoreciendo el intercambio gaseoso y las condiciones de oxigenación (Glaser et al. 2002; Laird et al. 2010).

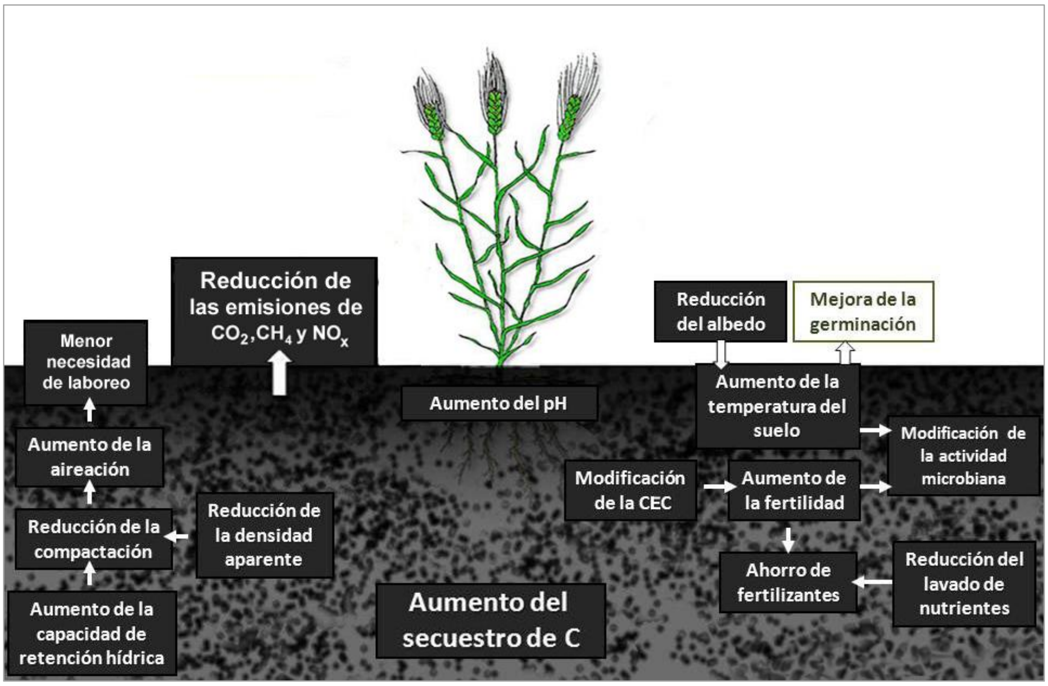

Figura 4. Resumen de los principales efectos del biochar sobre las características del suelo.

El biochar también puede alterar la fertilidad del suelo por medio de un aporte directo de nutrientes (Xu et al. 2013; Tammeorg et al. 2014) o aumentando la capacidad de intercambio catiónico (CEC) del suelo (Liang et al. 2006), lo que favorece la retención de nutrientes y evita pérdidas por lixiviación (Major et al. 2009; Yao et al. 2012). Por otro lado, los cambios del biochar sobre el pH y las condiciones redox del suelo, así como sobre la actividad biológica del suelo también pueden aumentar la disponibilidad de nutrientes para la planta (Chan y Xu 2009; DeLuca et al. 2009; Chintala et al. 2014).

El color oscuro que el biochar le confiere al suelo puede favorecer la absorción de las radiaciones solares y reducir el albedo, aumentando su temperatura (Genesio et al. 2012). Este aumento podría reducir la humedad, pero este efecto parece estar compensado por la alta capacidad de retención de agua del biochar (Laird et al. 2010; Genesio et al. 2012). El aumento de la temperatura del suelo junto con el aumento de la capacidad de retención de agua y del contenido de nutrientes tras la adición del biochar puede beneficiar a la germinación de las semillas y a la actividad microbiana (Downie et al. 2009). El biochar también puede resultar beneficioso como enmienda de suelos contaminados (Karamy et al. 2011). Numerosos estudios han demostrado la capacidad del biochar para adsorber física y químicamente pesticidas, herbicidas y metales pesados, reduciendo su disponibilidad para las plantas (Yu et al. 2009; Kookana et al. 2010; Beesley et al. 2010; Paz-Ferreiro et al. 2014).

Major et al. (2009) destacaron la necesidad de realizar una caracterización previa y completa del biochar, de modo que dependiendo del objetivo a conseguir y de las propiedades del suelo, sería aconsejable el uso de un determinado tipo de biochar. Por ejemplo, en suelos ácidos es aconsejable aplicar biochars con pH elevado, mientras que biochars con altos contenidos en C recalcitrante son recomendables si el objetivo principal es el secuestro de C.

Importancia de los rasgos funcionales para el análisis de los efectos del biochar sobre los cultivos

Las respuestas encontradas en la bibliografía de especies vegetales a la adición de biochar han sido generalmente positivas. Como ejemplo, Biederman y Harpole (2013) en un meta-análisis con 371 estudios encontraron que el biochar aumentó el crecimiento vegetal; sin embargo, los resultados eran muy variables dependiendo del tipo de biochar, tipo de suelo, clima y de la especie de estudio. También se han encontrado efectos negativos, así Gaskin et al. (2010) y Rajkovich et al. (2012) encontraron una reducción en el rendimiento del maíz con la adición de biochar. La mayoría de estos estudios coinciden en que esta variabilidad se debe principalmente a las condiciones experimentales de cada ensayo.

A la elevada variabilidad de los resultados obtenidos se debe sumar que la mayoría de estudios se han centrado fundamentalmente en el análisis de la biomasa y de la producción vegetal. Éstos no han entrado en detalle en los mecanismos específicos que subyacen a estas respuestas. Así por ejemplo, se ha profundizado muy poco en las respuestas de la raíz a las nuevas condiciones creadas en el suelo tras la adición de biochar (Prendergast-Miller et al. 2014). Es por tanto necesario el estudio de las respuestas de los diferentes órganos de la planta a la adición de biochar.

Los rasgos funcionales son atributos físicos y químicos de las plantas que pueden servir como indicadores o predictores de las respuestas de las plantas ante cambios en el ambiente (Lavorel y Garnier 2002; Cornelissen et al. 2003; Lopez-Iglesias et al. 2014), como pueden ser los cambios inducidos por el biochar sobre las características del suelo. En la Fig. 5 se indican los rasgos funcionales más importantes de los distintos órganos de la planta.

	Fracción de biomasa	Tamaño	Morfología	Composición química	Función
Fruto	FMF			Nutrientes	Reproducción
Hoja	LMF	Área foliar	SLA LDMC LRWC	Nutrientes	Fotosíntesis Transpiración
Tallo	SMF	Altura			Transporte de agua y nutrientes
Raíz	RMF	RL RD RLD	SRL TMDr RDMC	C/N	Captación de agua y nutrientes

Figura 5. Principales rasgos funcionales y variables evaluados en esta tesis doctoral. RMF, SMF, LMF y FMF: proporción de biomasa de raíz, tallo, hoja y fruto, respectivamente; RL: longitud de la raíz; RD: diámetro de la raíz; RLD: longitud de raíz por volumen de suelo; SRL: longitud específica de la raíz; TMDr: densidad tisular de la raíz; RDMC: contenido de materia seca de la raíz; SLA: área específica foliar; LDMC: contenido de materia seca de la hoja; LRWC: contenido relativo de agua de la hoja.

La raíz es la responsable de la adquisición de agua y nutrientes del suelo y su morfología está caracterizada por rasgos funcionales que reflejan la disponibilidad de recursos para la planta durante su desarrollo (Ostonen et al. 2007; Olmo et al. 2014). El rasgo morfológico más importante es la longitud específica de la raíz (SRL), que indica la longitud de raíz construida por unidad de biomasa de raíz y es considerado un buen indicador de los cambios en las características del suelo (disponibilidad de agua y nutrientes, pH, salinidad, etc.) (Ryser 2006; Ostonen et al. 2007; Olmo et al. 2014). Los cambios en la longitud específica de la raíz pueden deberse a su vez a cambios en el diámetro de la raíz (RD) y/o en la densidad tisular de la raíz (TMDr) (Ostonen et al. 2007). Por lo tanto, para entender los cambios en SRL deben estudiarse los efectos del biochar sobre RD y TMDr. Otros rasgos importantes relacionados con la raíz son la proporción de biomasa de la raíz (RMF), que indica la asignación de biomasa destinada a la raíz y la longitud de raíz por volumen de suelo (RLD), relacionada positivamente con la capacidad de exploración del suelo (Atkinson et al. 2000).

Los cambios en las características de la raíz pueden desencadenar cambios en la parte aérea de la planta. La hoja es el órgano de la parte aérea encargado de la fotosíntesis y la transpiración. Uno de los rasgos morfológicos más importantes de la hoja es el área específica foliar (SLA) que relaciona la superficie foliar con su masa y está relacionado con el crecimiento de la planta (Antúnez et al. 2001; Poorter y Bongers 2006). Otros rasgos funcionales importantes son el contenido relativo de agua de la hoja (LRWC) que es un indicador del estado hídrico de la planta (Slatyer y Taylor 1960) y el contenido de materia seca de la hoja (LDMC), asociado con la composición química de la hoja y con la fertilidad del suelo (Poorter y de Jong 1999; Hodgson et al. 2011).

Los rasgos funcionales que hemos descrito están muy relacionados con el crecimiento y la producción de la planta, de modo que su estudio puede aportarnos una mejor comprensión acerca de los mecanismos de respuestas de las plantas a la adición de biochar.

Importancia del estudio de la distribución del contenido de biochar en el suelo

Los ensayos con biochar en campo nos aportan una aproximación más realista de su impacto sobre las propiedades del suelo y el desarrollo de los cultivos. Desde un punto de vista práctico y económico, y considerando la naturaleza recalcitrante del biochar, no es necesario repetir la aplicación de biochar año tras año de forma continuada. De modo que evaluar el efecto residual de la aplicación de biochar a largo plazo adquiere una gran importancia en estudios sobre su efectividad agronómica.

Por otra parte, el biochar no es un material homogéneo y presenta un tamaño de partícula variable. El arado y la siembra del suelo, entre otras operaciones, dan lugar a movimientos en la capa superficial del suelo lo que puede originar un cambio en la distribución del biochar en el suelo. Existen pocos estudios que evalúen la movilidad del biochar en el suelo (Hammes et al. 2009; Foereid et al. 2011). En este sentido, Leifeld et al. (2007) concluyen que el arado a profundidades inferiores a 30 cm puede provocar que el biochar se movilice en el suelo de forma considerable. Estos movimientos podrían originar una distribución heterogénea del contenido de biochar en el suelo, sobre todo cuando el biochar se aplica en parcelas relativamente pequeñas.

La mayoría de estudios que evalúan los efectos del biochar a corto o medio plazo en condiciones de campo no suelen tener en cuenta la movilización del biochar, lo que puede dar lugar a interpretaciones erróneas. Este hecho hace necesario evaluar su incidencia sobre los resultados obtenidos y determinar el contenido y distribución de biochar una vez incorporado al suelo por medio de metodologías que sean rápidas, fiables, económicas y de fácil aplicación en campo.

Objetivos y justificación de la tesis doctoral

El objetivo principal de la investigación aquí descrita ha sido evaluar los efectos de la adición de biochar sobre las características del suelo y sus implicaciones sobre el crecimiento y la producción vegetal. Hasta la fecha, las revisiones bibliográficas que incluyen análisis cuantitativos de los resultados obtenidos en diferentes estudios han mostrado que los efectos del biochar sobre el crecimiento y la producción vegetal varían considerablemente, aunque predominan los efectos positivos (Jeffery et al. 2011; Biederman y Harpole 2013; Crane-Droesch et al. 2013; Liu et al. 2013). Generalmente, estos ensayos han usado un número limitado de especies diana y se han centrado fundamentalmente en el análisis de la biomasa y de la producción vegetal en su conjunto, sin entrar en detalle en los mecanismos específicos que subyacen a estas respuestas.

Para entender cómo el crecimiento y la producción de los cultivos pueden verse afectados por la adición de biochar es necesario explorar los rasgos morfológicos y fisiológicos implicados a nivel de todos sus órganos, incluido la raíz. En la Fig. 6 se muestra de modo esquemático los aspectos relacionados con las propiedades del biochar, las características del suelo y los mecanismos de respuesta de las plantas que se van a estudiar a lo largo de esta tesis doctoral.

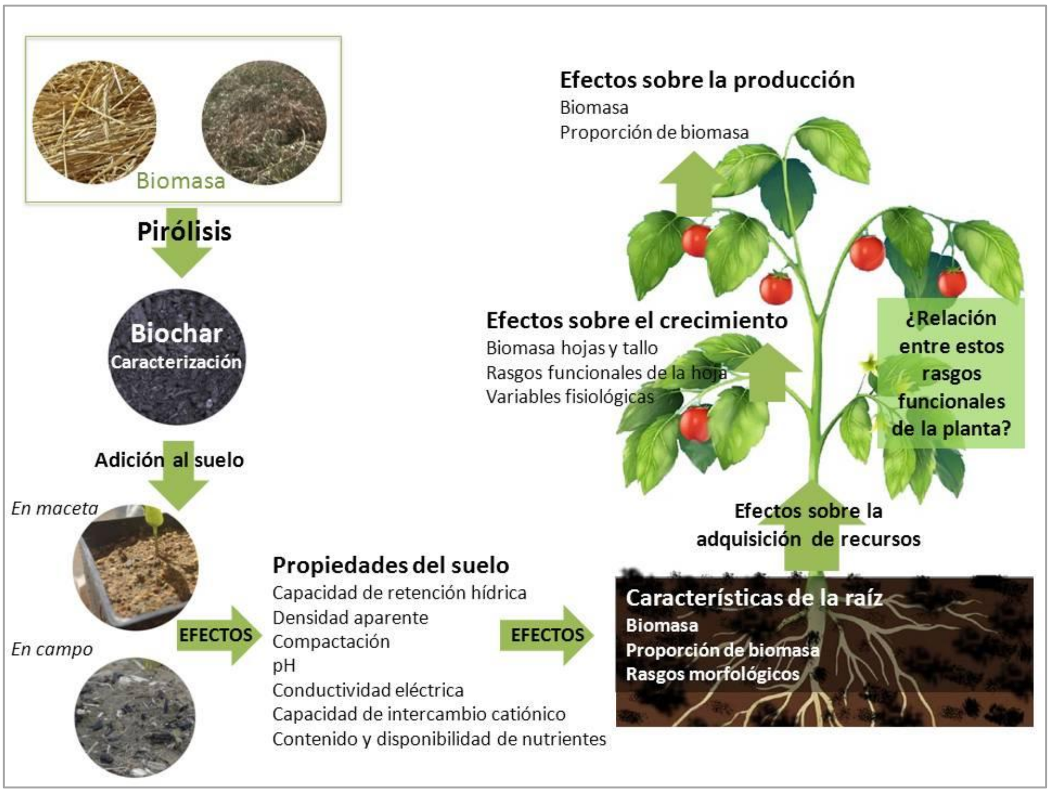

Figura 6. Síntesis de los principales procesos y mecanismos estudiados en esta tesis doctoral.

Para obtener unos resultados más completos se han combinado ensayos en condiciones controladas (cámara de cultivo e invernadero) con ensayos en campo. En los ensayos de campo se seleccionó el biochar procedente de poda de olivo basándonos en los buenos resultados obtenidos en los ensayos en condiciones controladas y su alto potencial de aplicabilidad en un futuro desarrollo industrial. El cultivo del olivo y la actividad de la industria extractiva del aceite de oliva tienen una gran importancia económica y social en Andalucía. La biomasa residual procedente del cultivo del olivo está actualmente infrautilizada debido a la escasa viabilidad económica que presentan las distintas alternativas de aprovechamiento existentes.

La presente tesis doctoral se estructura en 6 capítulos: introducción general, 4 capítulos experimentales y discusión general. Los objetivos específicos de cada capítulo experimental se detallan a continuación:

Experimentos en condiciones controladas

- En el capítulo 2, *"Changes in soil nutrient availability explain biochar's impact on wheat root development"*, los objetivos son evaluar los efectos de la adición de dos tipos de biochars de características diferenciadas (obtenidos a partir de poda de olivo y de paja de trigo), aplicados a diferentes dosis (con y sin fertilización), sobre las propiedades del suelo y las características de la raíz de trigo cultivado en maceta y cámara de cultivo.
- En el capítulo 3, *"Biochar affects fruit production in eight agronomic species through changes in root traits"*, los objetivos son conocer los efectos de la adición de un biochar (obtenido a partir de poda de olivo) sobre las propiedades del suelo, las características de la raíz y la producción, en un experimento con macetas en invernadero. En este ensayo se prestó especial atención a evaluar si los efectos sobre el cultivo se mantenían entre diferentes especies de interés agronómico. Para ello, usamos ocho especies: algodón, berenjena, colza, garbanzo, maíz, pimiento, tomate y soja.

Experimentos en campo

- En el capítulo 4, "*Wheat growth and yield responses to biochar addition under Mediterranean climate conditions"*, los objetivos son evaluar si los resultados obtenidos en experimentos bajo condiciones controladas son extrapolables en condiciones reales de cultivo. A tal fin, evaluamos los efectos de la adición de un biochar (obtenido a partir de poda de olivo) sobre el crecimiento y la producción de un cultivo de trigo en campo, centrando el estudio sobre los rasgos funcionales más importantes del cultivo.

- En el capítulo 5, “*Spatial heterogeneity of soil biochar content affects soil quality and wheat growth”,* los objetivos son conocer si un año después de la adición de biochar, los movimientos del suelo (arado y siembra) habían alterado y modificado el contenido y la distribución de biochar en el mismo; y evaluar los efectos de la heterogeneidad del contenido de biochar en el suelo sobre el crecimiento y la producción de un cultivo de trigo.

La presente tesis doctoral posee ciertos aspectos que pueden ser considerados como novedosos dentro del contexto de estudio de los efectos del biochar sobre el suelo, el crecimiento y la producción vegetal y que se detallan a continuación:

1) Combina estudios en cámara de cultivo, invernadero y en campo. Comprobar si son similares los efectos del biochar sobre el suelo y las plantas en condiciones controladas y en campo es de gran importancia para determinar si los resultados son generalizables.

2) Emplea diferentes especies de gran interés agronómico. Existen pocos estudios que estudien un amplio número de especies en condiciones experimentales similares. Una de las ventajas de los estudios multi-específicos es la obtención de resultados más completos y conclusiones más generales.

3) Estudio de una amplia cantidad de variables y rasgos funcionales. La mayoría de los estudios de los efectos del biochar sobre la producción vegetal se centran exclusivamente en el análisis de la biomasa y la producción. Sin embargo, para comprender cómo afecta el biochar a la producción vegetal es necesario evaluar su impacto sobre los rasgos morfológicos y fisiológicos más importantes de las plantas.

4) Estudio de los efectos del biochar sobre la raíz. No existen muchos estudios que evalúen la respuesta de la raíz a la adición de biochar. A lo largo de los experimentos se ha realizado un enorme esfuerzo con el fin de evaluar los efectos del biochar sobre la raíz de diferentes especies, incluyendo su estudio en campo.

5) Estudio de la distribución espacial en el suelo del biochar generada por el manejo del mismo. No existen apenas estudios que tengan en cuenta el grado de homogeneidad de la mezcla biochar-suelo durante los primeros años tras su incorporación al suelo. Así, se pretende evaluar cómo se ve afectada la distribución del biochar por los movimientos asociados al manejo y laboreo del suelo y cómo influyen estos cambios sobre los resultados que se obtienen en los ensayos experimentales.

6) Esfuerzo de síntesis de los resultados obtenidos. Se han propuesto variables como –efecto del biochar y se han mostrado correlaciones entre variables usando el gráfico de red de correlaciones para tratar de sintetizar los distintos procesos de las plantas y unirlos a través de un hilo conductor común: los rasgos funcionales.

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Capítulo 2. Changes in soil nutrient availability explain biochar's impact on wheat root development

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REGULAR ARTICLE

Changes in soil nutrient availability explain biochar's impact on wheat root development

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Abstract
Aims Biochar is a carbon-rich product obtained from the pyrolysis of organic materials. Its use, combined with fertilizers, can modify soil properties and affect root morphology and functioning. The main objective of this study was to evaluate the impact of biochar and fertilizer addition on root development and morphology.
Methods Durum wheat was pot-grown for 2 months using two types of biochar (produced from wheat straw and olive-tree pruning) applied at four rates (0, 0.5, 1 and 2.5 % *w*/*w*) and combined with three fertilization levels (0, 40 %-low and 100 %-complete).
Results Biochar addition at high rates increased the specific root length and decreased both root diameter and root tissue mass density, indicating a fine root proliferation, regardless of the fertilization level. This may have favoured the resource acquisition by increasing biochar-root interactions, soil exploration and the fertilizer efficacy. Biochar addition reduced N and Mn availability but increased P availability, which also influenced root growth.
Conclusions Changes in root morphology may therefore serve as an important indicator of soil changes induced by biochar and its study can contribute to a better understanding of the effects of the combined application of biochar and fertilizers on plant growth.

Keywords ammonium · nitrate · phosphorus · root traits · soil fertility · specific root length

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Introduction

Crop production to meet food demand depends on the use of fertilizers, particularly N and P, which can lead to negative environmental impacts due to over-fertilization and nutrient losses (eutrophication, soil and water pollution, greenhouse gas emissions, etc.) (Tilman et al. 2002; Conley et al. 2009; Cameron et al. 2013). Therefore, there is a need for reducing the environmental impact of fertilizers by using soil organic amendments which increase fertilizer use effectiveness and improve soil quality in the long-term (Diacono and Montemurro 2010; Lal 2015).

Recent interest in evaluating the use of biochar, charcoal or biomass-derived black carbon, as a valuable input for agriculture has been expressed by the scientific community (Lehmann and Joseph 2009; Barrow 2012). Biochar is defined as the carbonaceous product obtained when biomass is heated in an oxygen limited environment (pyrolysis) and it is attracting great attention as a

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mean for sequestering carbon due to its recalcitrant nature (Lorenz and Lal 2014; Jeffery et al. 2015). Other benefits can include the improvement of soil characteristics, such as a decrease in soil bulk density, and an increase in water holding and cation exchange capacity, favouring the retention of water and nutrients (Liang et al. 2006; Atkinson et al. 2010; Zheng et al. 2013). However, the large scale implementation of biochar as a soil amendment in agriculture is mainly limited by the cost of biochar production/application and the uncertainty of the benefits produced since they are highly depended on biochar type, application rate, soil type and fertility status, and environmental conditions (Crane-Droesch et al. 2013; Mukherjee and Lal 2014). Therefore, there is further need to elucidate the driving factors of plant growth response to biochar addition, especially those related to biochar-nutrient interactions (Chan et al. 2007; Schulz and Glaser 2012; Alburquerque et al. 2013). Biochar contains nutrients with a different degree of availability, which can act as a direct nutrient source (Lehmann et al. 2003). Also, biochar can alter nutrient availability (modifying soil pH, redox conditions, biological activity, etc.) and retain nutrient through cation exchange, surface interactions and water trapping processes (Mukherjee and Zimmerman 2013). All these processes are highly dependent on biochar type and soil properties and lead to complex biochar-nutrient interactions.

Most research on biochar has focused on aboveground plant biomass or production but much less on roots, due mainly to the difficulties in observing and studying the latter. This is surprising as roots are in direct contact with biochar, being the characterization of root-biochar interactions a key study in order to address the impact of biochar on plant resource acquisition.

Root structure can be described by several variables such as the specific root length (SRL) (Table 1). SRL is a root functional trait that characterizes the economic aspects of the roots (Eissenstat 1992; Ostonen et al. 2007). Maximizing SRL increases the root-soil interface and the root absorption potential (Eissenstat 1992). SRL is a root trait that includes the variation in root diameter (RD) and root tissue mass density (TMDr) (Ryser and Lambers 1995; Ostonen et al. 2007; Olmo et al. 2014a, b). TMDr is related with the life span and growth rate in roots of grasses (Ryser and Lambers 1995; Wahl and Ryser 2000) (Table 1). The mathematical decomposition of SRL (Ostonen et al. 2007) shows that it is equal to: $SRL = [1/(TMDr \times RD^2)] \times 4/\pi$. This shows that an increase in SRL could be due to a decrease in TMDr and/or RD. Therefore, to understand the causes of changes in SRL, the effects of biochar on both variables (RD and TMDr) have to be investigated. The above root traits can be interrelated with root elemental composition; in particular C and N concentrations, (Eissenstat et al. 2000; Valenzuela-Estrada et al. 2008; Comas and Eissenstat 2009). A high root C/N ratio, which expresses the ratio of tissue components related to structure (C) versus metabolism (N), would suggest a slower root activity. Also, C/N ratio can describe the soil N availability, and in turn, it is closely related with the plant nutritional status. Another relevant root variable is the root length density, which is related to the amount and rate of resource uptake (Atkinson 2000).

The present study builds upon a previous experiment (Alburquerque et al. 2013) where the effects of two

Table 1 The variables studied and their abbreviations, units, descriptions and functional roles based on the literature

Variable	Abrev.	Units	Description	Functional role
Root mass fraction		g root [g plant]$^{-1}$	Root biomass relative to total plant biomass	Resource allocation for root functions (uptake of water and nutrients)
Root length density	RLD	cm root cm^{-3} soil	Length of roots per unit volume of soil	Soil exploitation
Specific root length	SRL	m g^{-1}	Ratio of root length to root biomass	Related with root proliferation and resource acquisition ability
Root diameter	RD	mm	Mean root diameter	Related to root surface area, penetration ability and hydraulic conductivity of roots
Root tissue mass density	TMDr	g cm^{-3}	Ratio of root mass per root volume	Related to root dry matter content and the mechanical resistance of roots during soil exploration
C/N ratio	C/N		Ratio of carbon to nitrogen concentration	Trade-off in structural versus metabolic root function. An increase indicates lesser root activity

types of biochar (wheat straw and olive-tree pruning biochar), combined with three fertilization levels on the growth and yield of wheat were evaluated. The results showed that biochar addition had little effect on wheat yield in the absence of the fertilization. However, the combined addition of biochar and the highest fertilizer rate led to about 20–30 % increase in grain yield compared with the use of the fertilizer alone. In the present article, we address the impact of biochar addition on root growth. Our objective was therefore to evaluate the effects of different application rates of two biochar types on soil characteristics and wheat root variables, alone and combined with different levels of fertilization. We hypothesized that: (i) biochar addition alters soil physico-chemical properties and nutrient availability; (ii) these biochar-induced effects modify root morphology, favouring root proliferation; and (iii) as a result, both plant nutrient acquisition and the efficacy of the fertilizer added are increased.

Materials and methods

Two biochar samples were produced by slow pyrolysis using a laboratory-scale equipment based on the Anila Stove design (Iliffe 2009). The wheat straw biochar and the olive-tree pruning biochar (hereafter WS and OP Biochar, respectively) showed a maximum pyrolysis temperature of about 370 and 450 °C, respectively, and a total process time of about 4 h. Details of the biochar properties and analytical methods used are shown in Table S1 and Appendix S1 (Supplementary Information), respectively.

Biochars were added to a soil, classified as a Haplic Luvisol (IUSS Working Group 2006), collected from the surface horizon (0–20 cm) of an oak rangeland adjacent to the Rabanales Campus (Universidad de Córdoba; 37° 56′ 04″N, 4° 43′ 05″W, Córdoba, Spain). No tillage and no fertilizers had been applied in the last 5 years. The soil had a pH of 6.5 (1:2.5 soil/water ratio), and was characterized by a low fertility: loamy sand texture (80 % sand, 14 % silt, and 6 % clay), 9.0 g kg^{-1} total organic carbon, 0.8 g kg^{-1} total N, 12.5 mg kg^{-1} Olsen-P, and 10.1 cmol$_+$ kg^{-1} cation exchange capacity. Before starting the crop experiment, the impact of biochar addition on soil properties such as pH, electrical conductivity, bulk density, and field capacity was analyzed. Details of the analytical methods used for soil characterization can be found in Appendix S1 (Supplementary Information).

The plant growth experiment was carried out with durum wheat (*Triticum durum* L. cv Vitron) under controlled conditions in a growth chamber. For this, whea seeds were sown and five plants were grown in plastic pots (10 cm high and 7 × 7 cm) filled with 350 g dry soil or so l biochar mixture (see below). The average temperature of growth chamber was 23 °C, with a 16-h photoperiod, an average relative humidity of 42 %, and a light intensity of 250 μmol m^{-2} s^{-1}. The complete randomized design consisted of a factorial experiment with biochar application rate and fertilizer level as the main factors, and five repl - cates per treatment. We used four biochar application rates: 0, 0.5, 1, and 2.5 % *w/w* on a dry weight basis; and three levels of fertilization: F0 (without fertilization), F40 ('low'; 40 % of the optimal fertilization) and F100 ('complete'; 100 % of the optimal fertilization), providing a total of 0, 58 and 144 mL per pot during the crop cycle, respectively, of a full Hoagland nutrient solution. The optimal fertilization level (F100) was defined to satisfy crop needs (based on previous experiments) by considering the amount of nutrient extracted by the wheat crop in two months, nutr - ent sufficient ranges and number of plants per pot (Jones et al. 1991; Mengel et al. 2001). Besides the nutrien solution, deionized water was applied on a daily basis to keep soil moisture near 80 % of the water field capacity of soil and avoid leaching. The weight of each pot was registered daily to measure crop evapotranspiration during the experiment. This variable was calculated as the sum of water loss per pot due to evaporation and plant transpiration. To estimate soil N and P availability in the pots, anionic and cationic exchange resin membranes (I-100 and I-200 types, Electropure Excellion, Laguna Hills, CA, USA) were placed in the pots 5 cm below the so l surface. Ion resin membranes, known commercially as "root simulators" are considered to be a better proxy fo nutrient availability than soil nutrient pools (Qian and Schoenau 2002; D'Angelo et al. 2001).

Plants were harvested after 2 months. Plants were separated into aboveground and roots. Roots were gently washed from soil with tap water. Then, the whole roo system of the plants was placed on a scanner (Epson Perfection 836XL, Long Beach, CA, USA), in a transparent plastic tray filled with water. Root length, roo volume and RD were analyzed using WinRHIZO Pro 3.10 (Regent Instruments Inc.) (Himmelbauer et al. 2004). Finally, aboveground biomass and roots were oven-dried at 70 °C for at least 48 h, weighed and a roo subsample was ground in an agate mortar in order to determine C and N concentration using an elemental analyzer (Eurovector EA 3000, Milan, Italy). The following root variables were calculated: root mass fraction (root mass to total plant mass ratio, as percentage), SRL, TMDr and root length density. A summary of the different variables studied, with their description and functional roles, is given in Table 1.

Statistical analysis

For each biochar type, data were subjected to a two-way analysis of variance (ANOVA), considering biochar application rate and fertilization level as main factors. Data were transformed to fulfill the ANOVA assumptions [in WS Biochar treatments: RD and TMDr (log); resin-phosphate (square root) and in OP Biochar treatments: TMDr and resin-phosphate (log)]. Correlation and regression analyses were used to determine the relationships between variables. The programs STATISTICA™ v.8 (Statsoft, Inc., Tulsa, USA) and STATGRAPHICS Plus (Professional version, Windows 5.1; Statistical Graphics Corporation, Englewood Cliffs, New Jersey, USA) were used for statistical analysis.

Results

Effects of the biochar application

The characterization of the biochar-soil mixtures before cropping showed that both biochars affected soil properties. Overall, both biochars significantly increased soil pH and electrical conductivity (EC) (Supplementary Table S2). Furthermore, WS Biochar significantly decreased soil bulk density and increased soil field capacity at the highest biochar application rate (Supplementary Table S2). At the end of the crop experiment, resin-extractable nitrate and ammonium concentrations were decreased overall by OP and WS Biochar addition regardless of the fertilizer level applied, but resin-extractable phosphate concentration was increased (Supplementary Table S3).

Overall, the application of WS Biochar significantly decreased root biomass, but OP Biochar did not affect it (Table 2, Fig. 1a, b). In OP Biochar treated plants no significant changes were observed in root mass fraction, but WS Biochar reduced it (Table 2, Fig. 1c, d). Biochar addition to soil had opposite effects on root length density depending of the biochar type, increasing with OP Biochar addition and decreasing with WS Biochar addition (Table 2, Fig. 1e, f).

Root morphology was also affected by biochar addition (Table 2). When averaged over the fertilization level, OP Biochar addition greatly increased the specific root length (SRL, by 28 % at the 1 and 2.5 % rates compared to treatments without biochar addition; Fig. 2a), and decreased both root diameter (RD, by 8 % at the highest rate; Fig. 2c) and root tissue mass density (TMDr, by 18 % at the 1 and 2.5 % rates; Fig. 2e). In WS Biochar treatments, increasing biochar application rate decreased RD (by 8 % at the highest

Table 2 Main effects of biochar application rate (*B*) and fertilization level (*F*) on the studied root variables

Variable	Biochar application rate (B)		Fertilizer application rate (F)		$B \times F$		R^2	
	OP Biochar	WS Biochar	OP Biochar	WS Biochar	OP Biochar	WS Biochar	OP Biochar	WS Biochar
Root biomass	2.5 ns	22.4***(−)	56.1***(+)	49.8***(+)	13.6*	8.0*	72	80
Root mass fraction	3.1 ns	25.4***(−)	34.2***(−)	31.6***(−)	17.4*	6.9 ns	55	64
Root length density	31.2***(+)	6.0*(−)	35.3***(+)	58.1***(+)	15.1***	14.3**	82	78
SRL	45.3***(+)	25.1***(+)	1.0 ns	0.1 ns	7.7 ns	31.9***	54	57
RD	18.6**(−)	32.9**(−)	31.9***(+)	14.5*(+)	6.3 ns	7.8 ns	57	55
TMDr	39.2***(−)	0.9 ns	7.6 ns	21.1**(−)	5.9 ns	34.2**	53	54
C	9.2 ns	6.3 ns	0.3 ns	0.8 ns	7.7 ns	10.7 ns	17	18
N	2.9 ns	2.6 ns	5.8 ns	7.2 ns	11.2 ns	10.0 ns	20	20
C/N ratio	2.4 ns	1.3 ns	4.4 ns	7.6 ns	12.1 ns	11.7 ns	19	21

The proportion of the explained variance (SS_x/SS_{total}) and the level of significance (ns, not significant; * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$) for each factor and their interactions are indicated. R^2 is the percentage of total variance explained by the model. (+) or (−): positive or negative change for each factor. OP: olive-tree pruning, WS: wheat straw. SRL: specific root length, RD: root diameter, and TMDr: root tissue mass density

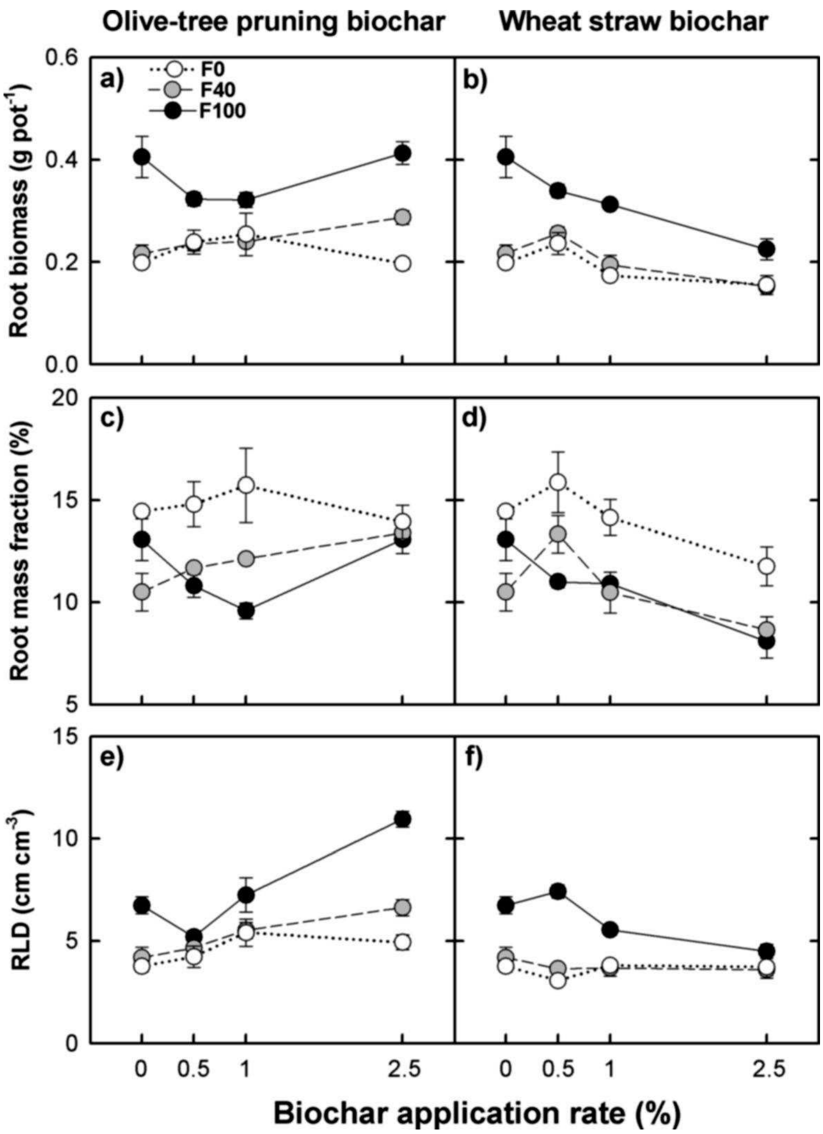

Fig. 1 Effects of biochar application rate and fertilization level on root variables for each type of biochar (mean value ± SE, *n* = 5; where absent, bars fall within symbols). RLD: root length density. F0: without fertilization, F40: fertilization 40 % and F100: fertilization 100 % (complete)

rate). High WS Biochar application rates generally led to the highest SRL and the lowest RD values irrespectively of the fertilization level applied (Fig. 2b, d). In contrast, root elemental composition (C, N and C/N ratio) was not significantly affected by biochar application (Table 2).

Effects of the fertilizer application

The fertilization significantly increased nitrate-extractable content, regardless of the biochar addition (Supplementary Table S3). In contrast, the fertilizer addition did not have a significant effect on ammonium or phosphate (Supplementary Table S3).

The fertilizer addition increased root biomass (Table 2, Fig. 1a, b), but reduced root mass fraction (Table 2, Fig. 1c, d). Similar to root biomass, t e fe tilizer addition significantly increased root length density (Table 2, Fig. 1e, f). The application of the fertilization had no effect on SRL (Table 2, Fig. 2a, b), but significantly increased RD in both biochars treatments (Table 2, Fig. 2c, d) and reduced TMDr in WS Biochar-treated plants (Table 2, Fig. 2f). In contrast, root elemental composition (C, N and C/N ratio) was not affected by the fertilization addition (Table 2).

Interactions between biochar and fertilizer application

In OP Biochar treatments, there was a significant biochar application rate (B) × fertilization level (F) inte action for root biomass, root mass fraction and root lengt density (Table 2). However, the B × F interaction fo t e

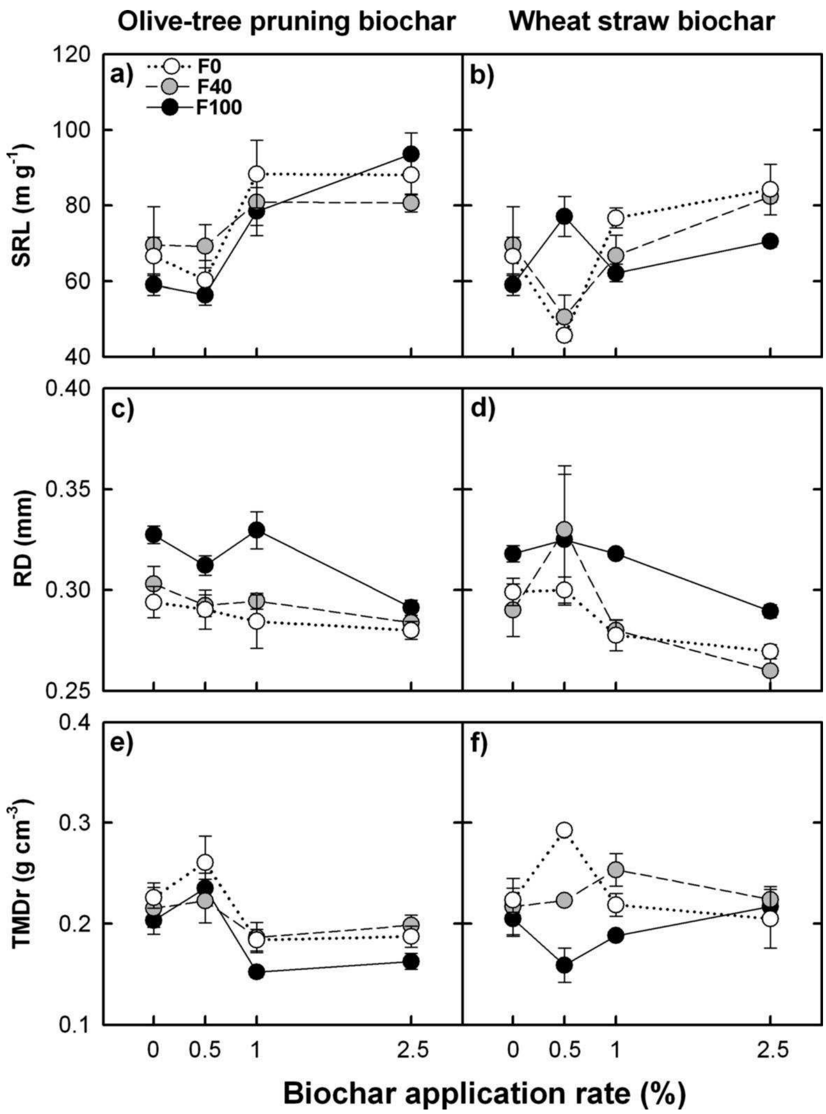

Fig. 2 Effects of biochar application rate and fertilization level on root variables for each type of biochar (mean value ± SE, *n* = 5; where absent, bars fall within symbols). SRL: specific root length, RD: root diameter and TMDr: root tissue mass density. F0: without fertilization, F40: fertilization 40 % and F100: fertilization 100 % (complete)

first two variables seems to be not important as in fact the biochar effect was not significant (Table 2). Therefore, root biomass increased and root mass fraction decreased as response to the use of the complete fertilization without a clear impact of biochar addition. In the case of the root length density, the significant B × F interaction is due to the fact that the highest increase in the root length density was obtained when biochar and fertilization were combined at the highest rates (Fig. 1e).

In WS Biochar treatments, there were significant B × F interactions for root biomass, root length density, SRL and TMDr. In the case of root biomass and root length density, the cause of the interaction is not due to a strong change in the effects of B and F, but to a high effect of biochar under complete fertilization (F100; Fig. 1b, f). With respect to root morphological traits, the cause of the interaction in the case of both SRL (Fig. 2b) and TMDr (Fig. 2d) seems to be the differences found when biochar was applied at 0.5 % depending on the fertilization level.

Relationships between root traits and nutrient availability

In OP Biochar treated plants, the SRL was negatively related with the availability of nitrate and ammonium, but not with phosphate availability (Fig. 3a, c, e). Similarly, we found a negative relationship between SRL and nitrate in WS Biochar treatments (Fig. 3b); meanwhile SRL was not affected by ammonium or phosphate availability (Fig. 3d, f). Our results show that the fertilizer addition did not affect the above relationships, which were mainly due to the biochar application rate, since high application rates of biochar led to a low N availability and high SRL

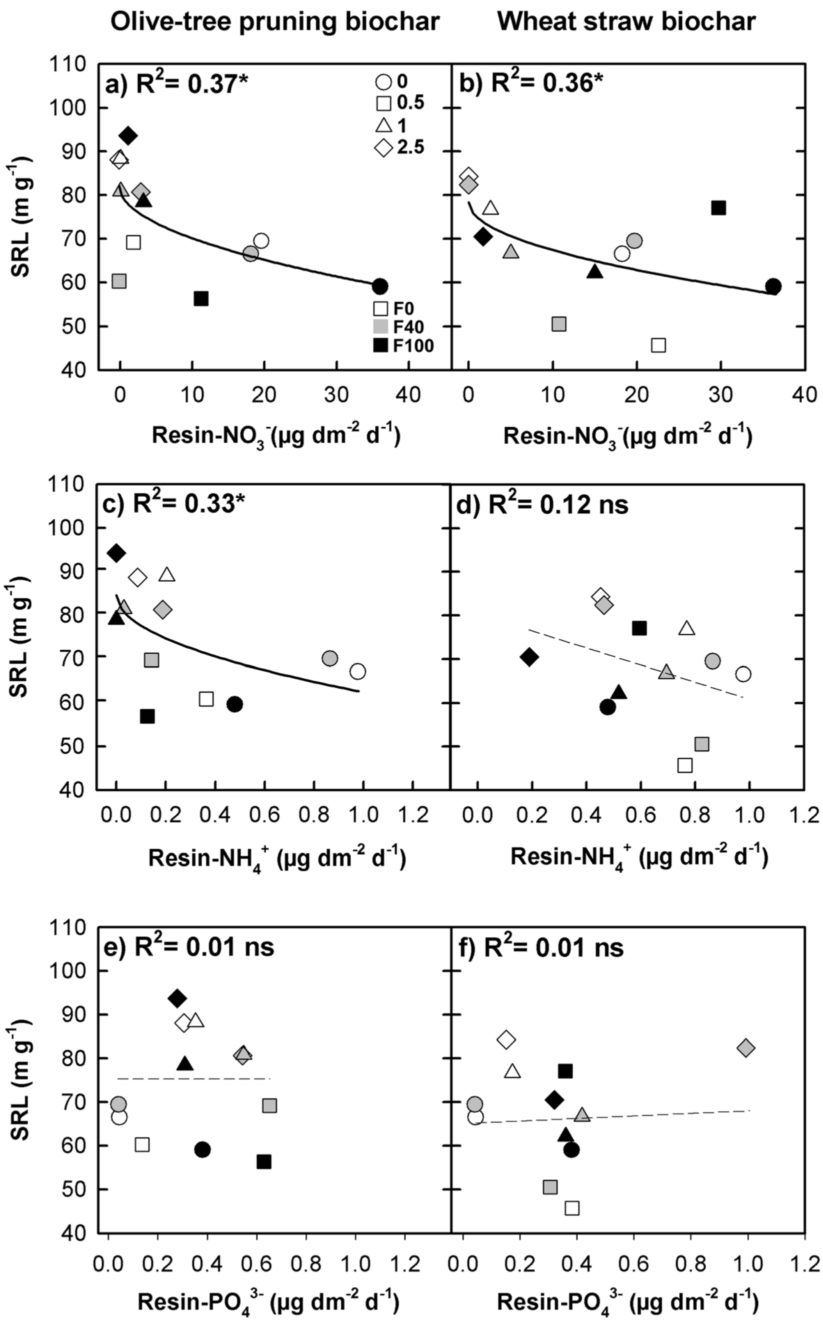

Fig. 3 Relationship between SRL (specific root length) and soil nutrients availability, for each biochar type. The different biochar application rates and fertilization levels are shown with different symbols and colors, respectively. F0: without fertilization, F40: fertilization 40 % and F100: fertilization 100 % (complete). Determination coefficients and significance levels are given: ns not significant, and *$P < 0.05$. The relationships of SRL with resin-extractable nitrate and -extractable ammonium were adjusted to square root curve

(Fig. 3). These changes in SRL were due to variations in either RD or TMDr. We found that the decrease in the resin-extractable nitrate content in OP Biochar treatments led to a decrease in the RD ($R^2 = 0.40$ $P < 0.05$, Supplementary Fig. S1a), while TMDr decreased as the resin-extractable ammonium content declined ($R^2 = 0.42$, $P < 0.05$; Supplementary Fig. S1g). In WS Biochar treatments, resin-extractable nitrate content was also related to RD ($R^2 = 0.63$, $P < 0.01$; Supplementary Fig. S1b). The resin-extractable phosphate content did not affect either RD or TMDr (data not shown).

Discussion

Biochar addition changes soil properties and nutri nt availability

Both biochars induced a liming effect on soil, increasing soil pH (Supplementary Table S2), which may affect nutrient availability. In addition, bioch r may modify soil nutrient availability by process s such as sorption, desorption and precipitation, which are also strongly influenced by changes in pH (Ch and Xu 2009; DeLuca et al. 2009; Chintala et al. 2014).

Both biochars enriched the soil in water-soluble mineral salts, increasing the soil electrical conductivity (Supplementary Table S2). This fact may have exerted a positive effect on soil nutrient status for some nutrients (for example, both biochars showed a high water-soluble K content, Supplementary Table S1). However, other nutrients such as ammonium and nitrate decreased their availability with the biochar addition (resin-extractable data). The porous nature of biochar, high surface area and ion exchange capacity determine the ability of biochar to sorb ammonium (through cation exchange) and nitrate (in solution in biochar pores) (Lehmann et al. 2003; Atkinson et al. 2010; Laird et al. 2010; Prendergast-Miller et al. 2014). These decreases were consistent with the reduction in the aboveground N concentrations (up to 33 %, Supplementary Fig. S2). We also expected that N availability reduction must also have decreased root N concentration; however, no significant effects were observed (Table 2). This could justify that even if aboveground plant N concentrations were reduced in biochar-treated plants, it did not greatly affect plant growth. In this sense, Prendergast-Miller et al. (2011) suggested that the presence of nitrate retained by biochar around roots may reduce the urgency for plant nutrient N uptake. On the other hand, plants in soil treated with biochar at the highest rate presented lower Fe and especially Mn aboveground plant concentrations (up to 62 and 82 %, respectively, Supplementary Fig. S2). The liming effect of biochar (especially of OP Biochar, richer in carbonates than WS Biochar) may lead to a decrease in the availability of these nutrients through precipitation reactions. The case of Mn was interesting as plants grown on the soil treated with high rates of biochar showed lower Mn concentrations than the sufficient level limit (Jones et al. 1991).

In contrast to the trend observed for N and Mn, the biochar addition (especially WS Biochar) increased P availability in soil. This resulted in increases in aboveground plant P concentration and plant P uptake (Supplementary Fig. S2), revealing a positive impact of biochar addition on plant P nutrition. This effect has been related to a direct supply of P in available forms (especially in ash-rich materials) and to changes in soil properties leading to P mobilization processes (Laird et al. 2010; Lehmann et al. 2003; Knowles et al. 2011).

Other physical soil properties were also modified by the biochar addition. The OP Biochar did not have a significant effect on soil field capacity nor soil bulk density, but WS Biochar addition determined an increase in soil field capacity and a decrease in soil bulk density (Supplementary Table S2). The improvement in these soil physical properties after biochar addition may favour root proliferation and promote crop development (Olmo et al. 2014a). Nevertheless, these changes seemed to be less relevant under our experimental conditions due to pots were irrigated daily, keeping soil moisture within the optimal range and avoiding leaching, and the sandy texture of the soil must have offered a low resistance to root penetration.

Biochar addition affects root development (biomass, partitioning and traits)

Our results demonstrated that biochar addition exerted a clear influence on root proliferation, but in some cases it depends on biochar type. The OP Biochar increased root length density (root length to soil volume ratio) but WS Biochar decreased it, especially at the complete fertilization (Fig. 1e, f). At the highest biochar application rate, plants treated with WS Biochar showed lower root biomass, root mass fraction (allocated less biomass to roots) and root length densities than plants in OP Biochar-treated soils. The reduction of root length density by WS Biochar addition was mainly explained by the reduction of root biomass (Table 2), since root length density and root mass fraction are interrelated and both variables describe root quantity and soil exploration (Atkinson 2000). This contrasting trend can indicate a lower resource supply in OP Biochar treatments regardless of the fertilizer addition, which led to develop a greater root system characterized by high root length densities that favours an intensive soil exploration (Guerrero-Campo et al. 2006). These results were corroborated by the impact of biochar on root morphology since both biochars led to an increase in the SRL, but more marked in OP Biochar treatments (Fig. 2a, b).

The SRL increase associated to biochar addition was due to a reduction of RD and TMDr (Supplementary Fig. S3). By increasing SRL, plants decrease the costs of root construction per unit of root length, and thus it enables the development of an extensive root system through fine roots (Ostonen et al. 2007; Olmo et al. 2014b). This favours water and nutrient acquisition and promotes crop development (Eissenstat 1992; Gahoonia and Niels 2004).

Several studies have reported that a SRL increase is the typical root response to conditions of low nutrient availability (Fitter 1985; Eissenstat 1992; Wahl and Ryser 2000). In this sense, we found that SRL correlated negatively with resin-extractable nitrate in OP and WS Biochar, and ammonium in OP Biochar (Fig. 3a, b, c). This was consistent with the detected decreases in resin-extractable ammonium and nitrate resulting from the addition of biochar (Supplementary Table S3), which suggest that both biochars showed a high retention capacity of N-forms.

The negative and significant regression found between aboveground Mn content in plants and SRL for OP Biochar (R^2 = 0.35, $P < 0.05$; data not shown), suggest that soil Mn availability could be another key factor to explain root response. Even though N and s ecially Mn concentrations were low, plants did not exhibited visual signs of deficiency and in fact biochar addition increased wheat grain yield, especially when it was combined with the fertilization (Alburquerque et al. 2013). Therefore, it seems that plants in biochar-treated soil get over these apparent nutrient limitations by modfying its root traits. This corroborates the hypotheses that (i) biochar addition modified soil nutrient availability, being the main driving factor to explain the impact of biochar on root development; and (ii) biochar modif ed root morphology through fine root proliferation.

With respect to the fertilizer addition, it affected, as expected, the root development (increasing root b omass, root length density and mean root diameter under both biochar types; Table 2, Figs. 1 and 2), which must be related to the higher plant growth as nutrient supply increased (Barraclough et al. 1989; Ryser and Lambers 1995; Ryser 1998; Dreccer et al. 2000). However, SRL was not affected by the fertilizer addition (Table 2). This null effect could be due to the contrary effect that fertilizer addition had on RD and TMDr, as fertilizer increased RD and decreased TMDr (Table 2).

Biochar addition improves fertilizer use efficiency

With respect to the nutrient-biochar interaction, biochar seems to act as a "sponge", as stated by previous authors (Schimmelpfennig and Glaser 2012; Clough et al. 2013), holding water and nutrients such as nitrate and ammonium and increasing P availability. This agrees well with Prendergast-Miller et al. (2014), who noted how roots are attracted towards biochar mainly due to the fact that biochar retained nitrate and supplied P. Therefore, an increase in fine root proliferation (a specific root length increase and RD and TMDr decreases) after biochar addition to soil seems to be a direct result of the root attraction by biochar, which maximizes biochar-root interactions and the access to the resources present in the biochar (Olmo et al. 2014a). Although an enhanced root-length production is effective at accessing immobile resources, the benefits of the interaction between biochar and roots will greatly depend on the availability of the nutrients retained in the biochar, as demonstrated by our results.

Under our experimental conditions where a poor nutrient soil was used, biochars had a low nutrient content and their addition decreased N and Mn availability and hence it could explain the limited impact of biochar on plant growth in the absence of the fertilization. However, the fine root proliferation induced by biochar may have improved the acquisition efficiency of the nutrients coming from the fertilization. These results seem to corroborate our third hypothesis and t could explain that the highest biochar application rate combined with the complete fertilization increased wheat grain yield by 33 and 22 % (OP and WS Biochar, respectively) with respect to the sole use of the complete fertilization, as noted in our previous study (Alburquerque et al. 2013).

To sum up, a better understanding of the processes involved in biochar's nutrient retention capacity and how they determine nutrient availability for plants s necessary to predict the biochar impact on nutrient use efficiency and strengthen beneficial effects on crop production. Due to the significance of N and P as plant nutrients and their potential negative environmental impacts, both N retention and P supply capacities of biochar offers attractive opportunities for sustainable agr - culture. Our results derive from the study of only two biochar types, and it would be necessary to evaluate more biochar types in order to corroborate and generalize the impacts on root development observed in the present study.

Conclusions

Biochar addition had contrasting effects on nutrient availability since it reduced N and Mn availability but increased P availability. These changes favoured fine root proliferation, increasing the specific root length and decreasing both root diameter and root tissue density, which seems to be driven by an attraction to biochar where water and nutrients are retained. These root changes seems to compensate the potential detrimental effects associated to low nutrient availability by enhancing soil exploration, interactions with biochar and increasing the fertilizers efficacy. Further research is needed to clarify the mechanisms involved in biochar's nutrient retention capacity and how they determine the ability of biochar to supply nutrients.

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Compliance with ethical standards This article does not contain any studies with human or animal subjects.

Conflict of interest The authors declare that they do not have conflicts of interest.

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Supplementary information

Table S1 Main characteristics of the two biochar types used in the experiment (mean value ± SE, n=2). OP: olive-tree pruning and WS: wheat straw. Significant differences are given (Student's t-test; ns not significant, a *P* <0.1, * *P* <0.05, ** *P* <0.01 and *** *P* <0.001)

Variables	**OP Biochar**	**WS Biochar**	***P***
Bulk density (g cm^{-3})	0.66±0.02	0.19±0.01	**
pH	11.51±0.13	10.66±0.02	*
Liming equivalence (g $CaCO_3$ kg^{-1})	219.4±11.5	70.6±2.6	**
Electrical conductivity (dS m^{-1})	1.5±0.1	2.8±0.1	**
Cation exchange capacity ($cmol_+kg^{-1}$)	21.0±0.6	47.4±0.9	**
Ash (g kg^{-1})	391.1±5.1	250.7±0.1	**
C_{org} (g kg^{-1})	483.9±0.2	627.9±0.5	***
Water-soluble C_{org} (mg kg^{-1})	2246±19	2138±18	ns
N (g kg^{-1})	9.6±0.5	8.3±0.1	ns
Water-soluble N (mg kg^{-1})	48±1	44±1	ns
P (mg kg^{-1})	834±19	845±46	ns
Water-soluble P (mg kg^{-1})	0.7±0.1	8.6±0.1	***
K (mg kg^{-1})	5420±10	7987±1	***
Water-soluble K (mg kg^{-1})	1364±22	6414±32	***
Ca (mg kg^{-1})	86041±92	5418±16	***
Mg (mg kg^{-1})	2020±7	1393±7	***
Fe (mg kg^{-1})	3028±55	1195±33	**
Mn (mg kg^{-1})	84±5	49±1	*
Zn (mg kg^{-1})	120±6	179±7	*
Cu (mg kg^{-1})	96±1	14±1	***
Germination index (lettuce, %)	86±8	56±1	a
Germination index (cress, %)	100±14	90±7	ns

Table S2. Effects of biochars applied at different rates on soil properties (mean value ± SE, n=5). OP: olive-tree pruning and WS: wheat straw. Significant differences are given (ns not significant, a *P* <0.1, * *P* <0.05 and *** *P* <0.001)

Application rate (%)	pH		Electrical conductivity (µS cm^{-1})		Bulk density (g cm^{-3})		Field capacity (%)	
	OP Biochar	WS Biochar	OP Biochar	WS Biochar	OP Biochar	WS Biochar	OP Biochar	WS Biochar
0	6.45±0.02		50±2		1.56±0.01		14.0±0.1	
0.5	7.52±0.03	6.65±0.08	60±2	48±3	1.59±0.03	1.58±0.01	13.1±0.3	13.9±0.1
1	7.89±0.02	6.92±0.10	76±2	58±3	1.63±0.01	1.57±0.02	13.7±0.1	14.9±0.1
2.5	8.23±0.01	7.62±0.06	104±3	70±4	1.58±0.01	1.49±0.01	14.3±0.2	15.8±0.1
P	***	***	***	***	a	*	ns	***

Table S3. Effects of biochar application rate (B: 0, 0.5, 1 and 2.5%) and fertilization level (F: 0, 40 and 100%) on resin-extractable soil nutrient concentration (µd dm^{-2} d^{-1}; mean value ± SE, n=5). OP: olive-tree pruning, WS: wheat straw. The effects of B and F and the interaction are shown at the bottom of the table. Significant differences are given (ns not significant, a *P* <0.1, * *P* <0.05, ** *P* <0.01 and *** *P* <0.001)

B	F	NH_4^+-N		NO_3^--N		PO_4^{3-}-P	
		OP Biochar	WS Biochar	OP Biochar	WS Biochar	OP Biochar	WS Biochar
0	0	0.82±0.11		11.44±4.27		0.04±0.02	
	40	0.72±0.16		23.07±3.38		0.06±0.02	
	100	0.63±0.08		36.23±13.58		0.21±0.13	
0.5	0	0.56±0.10	0.93±0.12	0.00±0.00	18.12±2.47	0.29±0.09	0.49±0.13
	40	0.19±0.09	0.95±0.11	2.31±0.85	8.58±3.51	0.47±0.18	0.27±0.02
	100	0.29±0.17	0.83±0.17	9.43±4.55	19.09±6.88	0.55±0.16	0.43±0.09
1	0	0.23±0.13	0.63±0.26	0.63±0.50	2.52±1.04	0.32±0.04	0.28±0.07
	40	0.07±0.05	0.72±0.14	0.15±0.15	4.65±2.02	0.38±0.21	0.29±0.11
	100	0.15±0.09	0.60±0.11	3.07±0.93	23.26±8.86	0.42±0.18	0.38±0.07
2.5	0	0.13±0.08	0.39±0.11	0.00±0.00	0.11±0.11	0.60±0.31	0.19±0.04
	40	0.11±0.11	0.39±0.13	1.81±1.31	0.00±0.00	0.49±0.22	0.76±0.15
	100	0.03±0.03	0.23±0.08	0.75±0.75	3.89±2.26	0.48±0.17	0.42±0.05
B		***	***	***	***	***	***
F		a	ns	*	**	ns	ns
B×F		ns	ns	ns	ns	ns	**

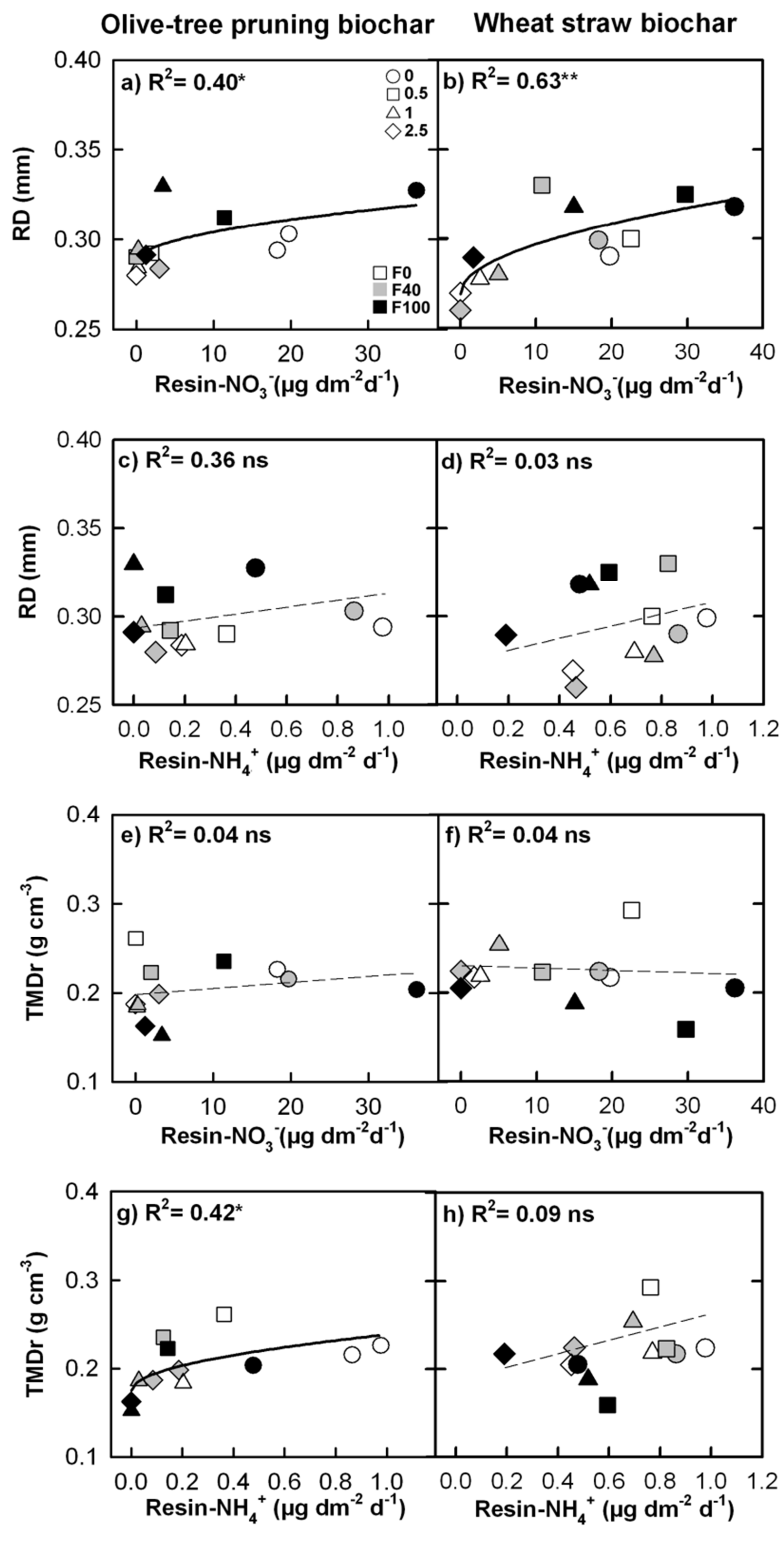

Fig. S1 Relationships between RD (root diameter) and TMDr (root tissue density), and soil nutrient availability, for each biochar type. The different biochar application rate and fertilization level are shown with different symbols. F0: without fertilization, F40: fertilization 40% and F100: fertilization 100% (complete). Determination coefficients and significance levels are given: ns not significant, * P <0.05 and ** P <0.01. The relationships between RD and resin-extractable nitrate, and between TMDr and resin-extractable ammonium were adjusted to exponential curves.

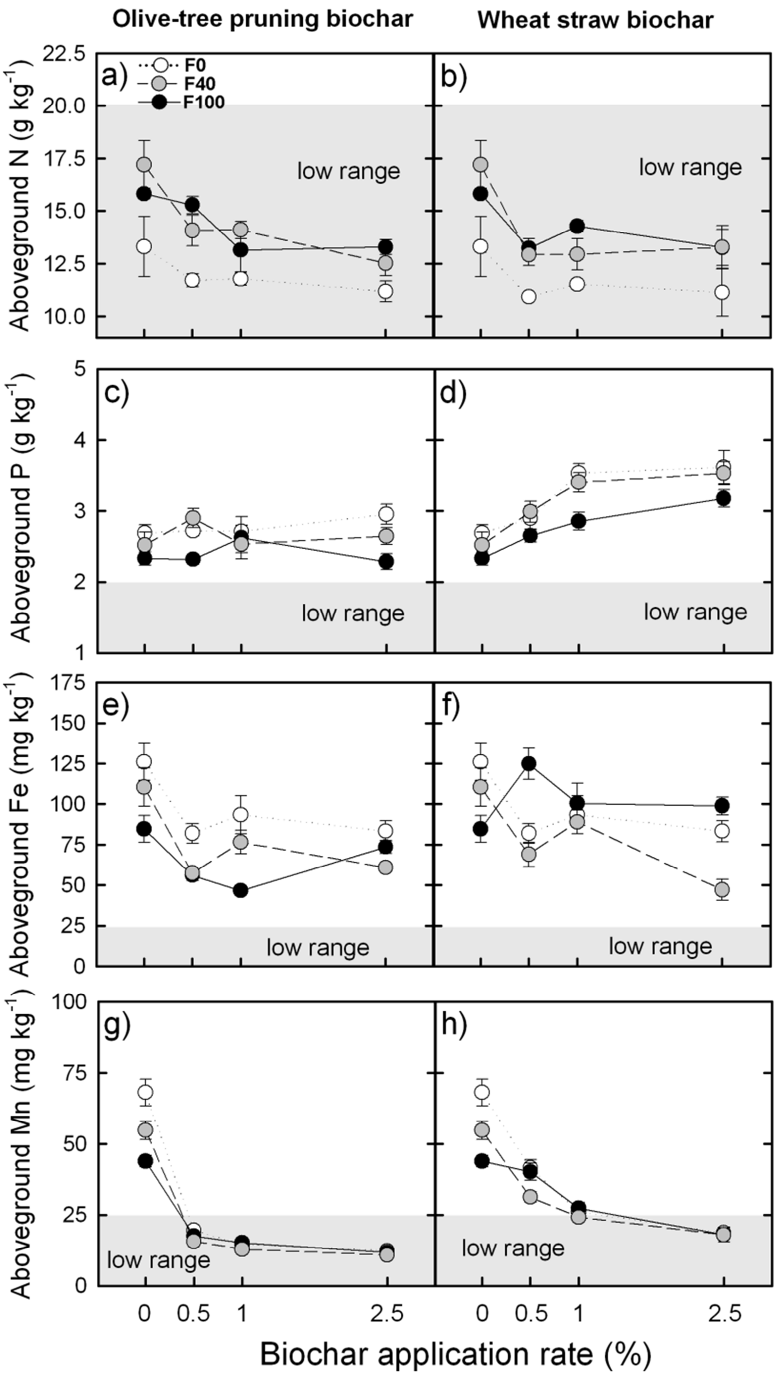

Fig. S2 Aboveground plant nutrient concentration (mean value ± SE, n=5; where absent, bars fall within symbols). F0: without fertilization, F40: fertilization 40% and F100: fertilization 100% (complete). Low ranges for wheat defined by Jones et al. (1991) are displayed in grey.

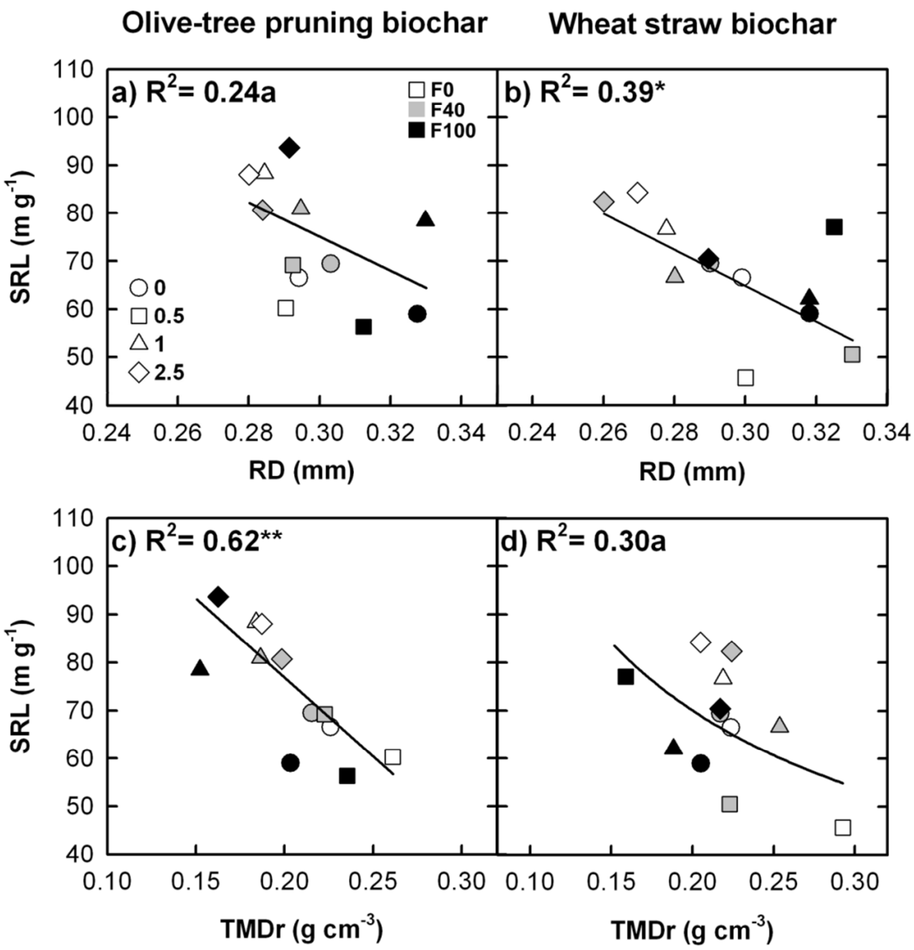

Fig. S3 Relationships between SRL (specific root length) and RD (root diameter), and TMDr (root tissue mass density) for each biochar type. The different biochar application rate and fertilization level are shown with different symbols. F0: without fertilization, F40: fertilization 40% and F100: fertilization 100% (complete). Determination coefficients and significance levels are given: a *P* <0.1, * *P* <0.05 and ** *P* <0.01). The relationship between SRL and TMDr, in wheat straw biochar treatment, was adjusted to an exponential curve.

Appendix S1

Analytical methods

The pH, electrical conductivity (EC), water-soluble C_{org}, N, P and K of the biochar samples were determined in the 1:10 (w/v) biochar/water extract after stirring the mixture mechanically for 2 h. The pH was measured with a pH meter with electrode glass in the supernatant, while EC (with a conductivity bridge), water-soluble C_{org} and N (with an automatic analyzer for liquid samples, TOC-V CSN+TNM-1 Analyzer, Shimadzu), water-soluble P (colorimetrically using a Lambda 35 UV/VIS Spectrophotometer, Perkin Elmer, Massachusetts, USA; Murphy and Riley, 1962) and water-soluble K (by atomic emission spectroscopy using a Jenway PFP7 Flame Photometer, Essex, UK) after centrifugation and filtering. The ash content was determined by a muffle furnace at 550 °C (TMECC 2002). The total organic carbon (after removal of inorganic C with diluted HCl) and total nitrogen were measured with an elemental analyzer (EuroVector, Milan, Italy). The biochar bulk density was estimated by weighing 10 mL of milled sample. The liming value of biochar was measured as calcium carbonate equivalency according to the 04.08-A method (TMECC 2002), where the biochar sample was treated with HCl in a 1:10 suspension and the unreacted acid is back titrated against standard NaOH. Total P, K, Ca, Mg, Fe, Mn, Zn and Cu were determined after dry ash sample digestion (04.12-C method; TMECC 2002). In the solution, P was determined colorimetrically (Murphy and Riley 1962), K by atomic emission spectroscopy, and Ca, Mg, Fe, Mn, Zn and Cu by atomic absorption spectrophotometry (AAnalyst 200 Atomic Absorption Spectrophotometer, Perkin Elmer, Massachusetts, USA). CEC was measured by a modified ammonium–acetate compulsory displacement method (Gaskin et al. 2008). The germination index was determined according to the method proposed by Zucconi et al. (1981) using cress (*Lepidium sativum* L.) and lettuce (*Lactuca sativa* L.). Two mL of biochar water-extracts (1:10 w/v filtered through 0.45 µm) was added to Petri dishes of 10 cm in diameter covered with filter paper and 8 seeds; 10 repetitions were performed per type of biochar. The seeds were incubated in darkness under controlled conditions (cress: 23 ^{o}C for 48 hours and lettuce: 17 ^{o}C for 120 hours). After this time, the number of seeds germinated and the length reached by the roots per plate was quantified. The results are expressed as germination index (GI), which is obtained by multiplying the percentage of germination (G), and the percentage of root growth (L), both with respect to control, and dividing by one hundred: GI (%) = (G × L)/100.

In the soil, pH and EC were determined in a 1:2.5 and 1:5 (w/v) soil/water extract, respectively. Particle size distribution was determined by the pipette method and $CaCO_3$ content was measured with a calcimeter. Field capacity was determined by placing soil in a 10 cm high cylindrical column, wetting it from the top by adding water very slowly to avoid trapping air in the soil column, letting it drain for 48 h, and then measuring gravimetrically the water content at a depth of 3−6 cm. The bulk density of the soil was determined by weighing 1000 mL of dry soil. The total organic carbon and total nitrogen were measured with an elemental analyzer (EuroVector, Milan, Italy). The soil available P was extracted with 0.5 M $NaHCO_3$ buffered at pH 8.5 (1:20, w/v) for 30 min and measured colorimetrically ("Olsen P"; Olsen and Sommers 1982). The CEC was measured by saturation with sodium at pH 8.5, which included washing with sodium acetate, ethanol, and 1 M ammonium acetate. Resin-extractable nitrate, ammonium and phosphate were extracted from the resin membranes by shaking them in 50 mL of 2 M KCl for 1 h at 200 rpm in an orbital shaker. The concentration of NH_4^+-N and NO_3^--N in the extract was measured by colorimetry (indophenol blue method) using a microplate reader (Sims et al. 1995), while that of PO_4^{3-}-P by the molybdenum blue method using a microplate reader (D'Angelo et al. 2001).

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Capítulo 3. Biochar affects fruit production in eight agronomic species through changes in root traits

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REGULAR ARTICLE

Biochar affects fruit production in eight agronomic species through changes in root traits

Manuel Olmo · Rafael Villar

Abstract

The use of biochar (BC) as a soil amendment has attracted much interest with regard to increasing soil carbon sequestration, improving soil fertility, and increasing crop production. However, studies to date show a great variability in their results, depending on the plant species studied. Moreover, the effects of BC addition on the roots are not well understood.

We investigated the effects of a BC, obtained from olive-tree prunings and applied to soil at 5% w/w, on soil properties, root morphology, and crop yield in a greenhouse pot experiment. We used eight species of agronomic interest: *Brassica napus* L., *Capsicum annuum* L., *Cicer arietinum* L., *Glycine max* L., *Gossypium herbaceum* L., *Solanum lycopersicum* L., *Solanum melongena* L., and *Zea mays* L.

Addition of BC reduced soil bulk density and increased both soil water content and nutrient availability, as well as increasing the specific root length. It also increased aboveground biomass, specific leaf area, leaf relative water content, and both seed and fruit production, the latter being positively correlated with the specific root length changes.

Most species responded positively to the addition of BC derived from olive-tree prunings, the response being directly related to changes in root traits.

Key-words agricultural wastes, specific leaf area, soil fertility, specific root length, functional traits, yield

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Introduction

Biochar (BC) application to soil is being increasingly investigated as a soil improvement and carbon (C) sequestration strategy (Stavi and Lal 2013; Lal 2015). BC is a product obtained from the thermo-chemical decomposition of organic matter in the absence of oxygen (pyrolysis) (Lehmann et al. 2006). The pyrolysis process stabilizes the C existing in the organic matter into a product more resistant to chemical and biological decomposition. Therefore, when BC is incorporated into the soil the C is kept stable for longer and is not emitted to the atmosphere, as would occur with the biomass decomposition (Stavi and Lal. 2013; Lehmann and Joseph 2015). BC may enhance soil fertility directly, by providing nutrients (Xu et al. 2014; Tammeorg et al. 2014), or indirectly, by modifying nutrient availability and increasing fertilizer efficiency (Alburquerque et al. 2013). The direct impact of BC application to soil is exerted by BC’s high pH, porosity, specific surface area, and cation exchange capacity (Downie et al. 2009; Sohi et al. 2010; Singh et al. 2010; Van Zwieten et al. 2010).

Plant responses to the addition of BC to soil can vary greatly (see the reviews of Jeffery et al. 2011; Biederman and Harpole 2012; Liu et al. 2013). Although the responses to BC addition have been mostly an increase in production (Atkinson et al. 2010; Jha et al. 2010; Kookana et al. 2011; Olmo et al. 2014), no effects (Spokas et al. 2012; Nzanza et al. 2012; Zhang et al. 2013) and negative effects (Gaskin et al. 2010; Rajkovich et al. 2012) have also been reported. The high variability in plant responses mainly depended on the BC type, application rate, soil characteristics, and plant species (see reviews by Jeffery et al. 2011; Biederman and Harpole 2012; Liu et al. 2013).

Generally, the increase in crop production after BC addition is positively related to soil quality improvements, such as increases in water retention capacity (Baronti et al. 2014; de Melo Carvalho et al. 2014; Olmo et al. 2014a) and nutrient availability (Lehmann et al. 2003; Clough et al. 2013; Ventura et al. 2013). The meta-analysis of Crane-Droesch et al. (2013) found that the increases in ion exchange capacity and soil pH after BC addition are the main factors that affect crop production, especially in poor and acid soils. The positive relationship between improved soil quality and crop production after BC addition is well known; however, the mechanisms underlying plant responses to BC have not been studied in depth. For example, the responses of roots to the new conditions created in the soil after BC addition have been scarcely explored (but see Prendergast-Miller et al. 2014; Olmo et al. 2014a) and little is known about the influence of root-BC interactions on plant production.

Some morphological characteristics of roots may reflect the availability of resources for plant growth (Ostonen et al. 2007; Franco et al. 2011). The specific root length (SRL) is the most frequently measured morphological trait of roots. The SRL characterizes the economic aspects of the roots and reflects environmental changes (Ryser 2006; Ostonen et al. 2007; Olmo et al. 2014b). The SRL depends on two variables: the root tissue mass density (TMDR) and root diameter (RD) (Ostonen et al. 2007; Olmo et al. 2014b). An increase in SRL could be due to a decrease in TMDR and/or RD. Therefore, to understand the causes of changes in SRL, the effects of BC on both variables (RD and TMDR) have to be investigated. Changes in root morphology could be important in order to gain rapid access to soil nutrients and water, leading to changes in aboveground functional traits. In this sense, at the leaf level, the specific leaf area (SLA) is a key trait related to light capture, photosynthetic capacity, and plant growth rate (Antúnez et al. 2001; Wright et al. 2004; Poorter et al. 2009). Other important leaf traits related to soil fertility and water status are the leaf dry matter content (LDMC) and leaf relative water content (LRWC) (Cunningham et al. 1999; Hodgson et al. 2011). The study of these functional traits (at root and leaf level) could be a useful approach to better understand the effect of BC on plant productivity.

Given the scarce number of studies on the effects of BC on multiple plant species, we evaluated in the present study the impact of BC produced from olive-tree prunings on eight species of great agronomic interest. We used this type of BC because olive-tree pruning residues are produced in large quantities in the Mediterranean region (about 2 million tons year^{-1} in Andalusia; AAE 2015) and their pyrolysis to produce BC can be a reliable solution to the management of these residues.

The objectives of this study were: (1) to determine how BC affects important soil variables (soil water content, bulk density, pH, nutrient availability, etc.); (2) to show how changes in soil properties after BC addition affect root characteristics (key root morphological traits) and aboveground plant development (biomass, partitioning, and traits), and; (3) to explain how plant trait changes may determine the plant yield. We hypothesized that: (i) application of BC enhances soil quality; (ii) plants respond to these soil improvements by favoring root proliferation, and; (iii) these root adjustments lead to increases in plant growth and crop production.

Materials and Methods

Biochar and soil

The olive-tree pruning residues were provided by “Valoriza Energía Puente Genil” (Córdoba, Spain) and were pyrolyzed in a pilot plant at 450 °C by slow pyrolysis at the University of León (Natural Resources Institute, Spain). The BC was ground with a stainless steel mill to <2 mm. It showed the following characteristics: pH of 9.5, electrical conductivity (EC) of 1.4 dS m^{-1} (1:10 BC: water), cation exchange capacity of 29.6 cmol$_+$ kg^{-1}, 642.2 g kg^{-1} organic C, 12.1 g kg^{-1} total N and 0.8, 5.4, 86.0, and 2.0 g kg^{-1} of P, K, Ca, and Mg, respectively. Details of the analytical methods for BC characterization can be found in Alburquerque et al. (2014).

The soil used in this study, classified as a Haplic Luvisol (IUSS Working Group, 2006), was collected from the surface horizon (0-20 cm) of a field adjacent to the Rabanales Campus (University of Córdoba; 37°55'51" N, 4°43'16' W, Córdoba, Spain). The soil is a sandy-clay loam (55% sand, 14% silt, and 31% clay), with a pH of 7.97 (1:2.5 soil/water ratio), EC of 0.09 dS m^{-1} (1:5 soil/water ratio), 413 g kg^{-1} calcium carbonate equivalent, 4.1 g kg^{-1} total organic-C content, and 11.9 mg kg^{-1} Olsen-P. The concentrations of K, Ca, Mg were 0.19, 16.7, and 0.24 g kg^{-1}, respectively. The concentration of Mn, Fe, Cu and Zn was 9.59, 1.2, 0.96 and 0.92 mg kg^{-1}, respectively. Details of the analytical methods used for soil characterization can be found in Appendix S1 (Supplementary Information).

Experimental design

The experiment consisted of a factorial completely randomized design with two factors: BC treatment and plant species. The BC was added to the soil at 5% w/w, based on previous experiments (Alburquerque et al. 2013), there was also a control without BC addition. Eight agronomic species were selected: *Brassica napus* L. (rapeseed), *Capsicum annuum* L. (pepper), *Cicer arietinum* L. (chickpea), *Glycine max* L. (soybean), *Gossypium herbaceum* L. (cotton), *Solanum lycopersicum* L. (tomato), *Solanum melongena* L. (eggplant) and *Zea mays* L. (corn). Hereafter, we refer to the species by the genus name and the first letter of the species name (for example *Brassica n.*). We used seven replicates per species and treatment. Pots with a volume of 1 L (20 cm high and 9 cm × 9 cm) were filled with 1150 g of dry soil or soil-BC mixture. To prevent loss of soil through the drainage holes of the pots, small plastic nets were placed in the bottom of the pots.

The experiment was established on March 25, 2014. To ensure the supply of seedlings, four seeds of each species were sown per pot. Five days after emergence, only one seedling was kept per pot. The plants were initially grown in an open courtyard at the University of Córdoba (Córdoba, Spain, 37°55'08" N, 4°43'28' W), with an average temperature of 22.9 ± 3.7 °C (mean ± SD) and a relative humidity of 64.7 ± 10.1% (Central Research Support Service, SCAI at the University of Córdoba). The plants were watered every day to the field capacity of the soil. The pots were rotated every two weeks to avoid the influence of possible microclimate variability. On June 10, 2014, the plants were transferred to a greenhouse at the University of Cordoba, having more favorable environmental conditions for their development and fruit production. The pots were placed randomly. During this phase the temperature in the greenhouse was 28.3 ± 6.3 °C (mean ± SD), relative humidity was 54.4 ± 17.9 % (data logger PCE-HT71, PCE-Instruments, Tobarra, Spain) and solar radiation reaching the plants was 375 ± 114 µmol photons m^{-2} s^{-1}, measured with a photometer (EMS7, PP-system, UK) on a clear day (July 16, 2014 at 12:00 PM Solar time). The plants were watered every two days to field capacity, leaving the pots to drain freely. To prevent nutrient deficiencies, a liquid fertilizer (7:3:5 N:P:K with 0.05, 0.02, 0.01, 0.002 and 0.001% of Fe, Mn, B, Cu and Zn, respectively; COMPO ® green plants) was added with the irrigation water, at a concentration of 5 mL L^{-1} water. The weight of two pots per species and treatment was taken weekly to calculate the soil water content as the difference between the wet mass of soil and the initial dry mass of the soil in the pot.

The plants were harvested on July 15, 2014. Plant height was measured and each plant was extracted from its pot and separated into the aboveground part and roots. The roots were washed carefully with tap water to avoid loss of roots. Four plants per species and treatment were randomly selected for root measurements. From each of the four selected individuals we took a sub-sample of fine roots (diameter <0.5 mm) to be scanned. We chose these roots for their important role in the exploration of the soil for water and nutrient uptake (Eissenstat 1992; Ostonen et al. 2007). This fraction comprises about 20 % of the root biomass. The fresh mass was taken and the roots were placed on a scanner in a transparent plastic tray filled with water. Root length (RL, m), root diameter (RD, mm) and root volume (cm^3) were analyzed using WinRHIZO Pro v.3.10b (Regent Instrument Inc., Quebec, Canada). Finally all the roots were dried at 70 °C for 48 h and the dry mass was taken. The specific root length (SRL, m g^{-1}) was calculated as root length divided by root dry mass, the root tissue mass density (TMDR, g cm^{-3}) was calculated as root dry mass divided by root volume, and the root dry matter content (RDMC, g g^{-1}) was calculated as root dry mass divided by fresh mass.

The aboveground part was separated into stems, leaves, and fruits. A subsample of leaves per plant was selected to measure the leaf functional traits. This leaf subsample (approximately 20% of the leaf biomass) was separated into blade and petiole. In the case of compound leaves, the leaflets were separated. The fresh mass of leaf blades, petioles, leaflets and rachis was taken. The blades and leaflets were scanned (ADF HP Scanjet 6300c-Hewlett-Packard, Colorado, USA) and leaf area (LA, m^2) was calculated using Image Pro 4.5 (Media Cybernetics Inc.). Afterwards, the leaves, blades and leaflets were wrapped in moist paper and left in plastic bags at 4 °C for 24 h to fully rehydrate them; then, the saturated fresh mass was taken. Finally, the dry mass of all these fractions (70 °C, 48 hours) was taken. The following leaf traits were calculated: specific leaf area (SLA, LA divided by the dry mass of the blade or leaflets, m^2 kg^{-1}), the leaf dry matter content (LDMC, the dry mass of blade or leaflets divided by the fresh saturated mass, g g^{-1}), and the relative water content [LRWC, 100 × (fresh mass - dry mass) / (saturated mass - dry mass), g g^{-1}]. The proportions of leaf biomass (LMF), stem (SMF), root (RMF), and fruits (FMF) were calculated, respectively, as the dry mass of leaves, stem, root and, fruit divided by the total plant dry biomass (g g^{-1}).

Data and statistical analysis

To detect if the BC treatment and plant species had some effect on the variables studied, the data were analyzed with a two-way ANOVA, with species and treatment (BC and control) as factors. To analyze differences between the treatments, a Tukey post-hoc test with a *P* level of 0.05 was used. The data were transformed (log) if variables did not fulfill the assumptions of the ANOVA. Pearson correlations were used to analyze the relationships between variables. For statistical analysis, STATISTICA ™ v.8 software (StatSoft, Inc., Tulsa, USA) was used.

As we used many species with very different characteristics, to understand better the effect of BC on different variables it was necessary to study the relative effects of BC. This has been done in other studies (see Liu et al. 2013) considering the response ratio, or "*BC effect*", as:

BC effect on variable $X = X_T / X_C$ (*Equation 1*)

where X_T is the mean value of variable X in the treatment with BC and X_C is the mean value of variable X in the control treatment.

A value of the BC effect > 1 means that the addition of BC increased the value of the variable, and the contrary for a value < 1. Therefore, this calculation expresses the effect of BC (increase or decrease) on different variables and its magnitude. For plant variables, we calculated a BC effect for each species and variable. These calculations will allow us to summarize the effects of BC on the most important variables of the study (soil and plant traits).

Results

Effects of biochar on soil and root characteristics

The characterization of soil samples (control and BC treatment) before planting showed that BC addition affected significantly the soil characteristics (Table 1). Thus, BC reduced soil bulk density (from 1.62 to 1.55 g cm^{-3}) and significantly increased pH (from 8 to 8.5), EC (from 85 to 136 µs cm^{-1}), and nutrient concentration (Ca, K, Mg, P, Cu and Mn) (Table 1). Addition of BC also increased the soil water content (on average, by 9%; Table 1), although statistically significant differences were only observed on a few dates (Supplementary Fig. S1).

The species greatly differed in their root characteristics (Table 2), for example, in the RMF (Table 2, Fig. 1a). Also, SRL showed a high variation between species (Table 2), with a minimum values of 105 ± 30 m g^{-1} (*Solanum m.*) and a maximum of 324 ± 57 m g^{-1} (*Brassica n.*, Fig. 1b). The addition of BC significantly affected the root variables (Table 2), it reduced RMF (from 0.28 to 0.23 g g^{-1}; Fig. 1a) and increased SRL (from 179 to 208 m g^{-1}; Fig. 1b). However, not all species responded in the same way, as indicated by the significant species (SP) × biochar (BC) interaction for SRL (Table 2). The BC addition reduced SRL (but not significantly) in some species; increased it (not significantly) in others; and increased it significantly in *Brassica n.* (Fig. 1b). BC significantly reduced TMDR (from 0.14 to 0.12 g cm^{-3}; Table 2, Fig. 1c) with a significant SP × BC interaction (Table 2). BC reduced TMDR (not significantly) in some species, but it increased it (not significantly) in other species (Fig. 1c), and did not have any effect on RD or the root dry biomass (Table 2, Fig. 1d).

Effects of biochar on aboveground traits, biomass and fruit production

Similar to the root traits, we found that the species greatly differed in their aboveground variables and leaf traits (Table 2). For example, we found significant differences in aboveground biomass between species (Table 2), *Capsicum a.* showing the lowest value (2.6 ± 0.3 g) and *Zea m.* the highest (10.9 ± 1.3 g) (Fig. 2a).

Table 1. Mean values ± SE (n= 4) of the soil variables analyzed, for the control and biochar treatments, at the beginning of the experiment.

Variable	**Control**	**Biochar**	***Significance***
Soil water content (g g^{-1})	0.23 ± 0.01	0.25 ± 0.01	**
Bulk density (g cm^{-3})	1.62 ± 0.01	1.55 ± 0.01	***
pH	7.97 ± 0.03	8.46 ± 0.04	***
EC (µS cm^{-1})	85.03 ± 2.14	136.15 ± 4.86	***
Olsen-P (mg kg^{-1})	11.88 ± 0.73	20.29 ± 1.08	***
NH_4OAc-K (g kg^{-1})	0.19 ± 0.01	0.81 ± 0.04	***
NH_4OAc-Ca (g kg^{-1})	16.66 ± 0.25	18.41 ± 0.59	*
NH_4OAc-Mg (g kg^{-1})	0.24 ± 0.01	0.29 ± 0.01	*
EDTA-Cu (mg kg^{-1})	0.96 ± 0.03	1.26 ± 0.05	**
EDTA-Mn (mg kg^{-1})	9.59 ± 0.62	16.61 ± 1.03	**
EDTA-Fe (mg kg^{-1})	1.17 ± 0.05	1.32 ± 0.11	ns
EDTA-Zn (mg kg^{-1})	0.92 ± 0.04	1.03 ± 0.08	ns

EC electrical conductivity. The significance is shown as: *ns* not significant; * $P<0.05$; ** $P<0.01$; and *** $P<0.001$.

Table 2. Results of the two-way ANOVA in which the effects of Species (SP), Biochar (BC), and their interaction (SP × BC) on the different variables studied are shown.

	Factors			
Variable	**Species (SP)**	**Biochar (BC)**	**SP × BC**	R^2
Root				
Root biomass	74.2***	0.1 ns	1.1 ns	70***
Root mass fraction (RMF)	59.7***	7.8*** (-)	1.4 ns	62***
Root dry matter content (RDMC)	30.7***	0.1 ns	9.2 ns	25**
Specific root length (SRL)	63.1***	3.4** (+)	13.5***	73***
Root tissue density (TMDR)	24.3***	4.1*(-)	16.5*	30**
Root diameter (RD)	65.8***	0.1 ns	2.1 ns	59***
Aboveground				
Height	91.4***	1.4*** (+)	0.6 ns	92***
Aboveground biomass	70.8***	6.0***(+)	1.1 ns	74***
Plant biomass	74.4***	3.2** (+)	0.9 ns	74***
Stem biomass	79***	2.5** (+)	2.0 ns	80***
Stem mass fraction (SMF)	81.5***	0.2 ns	2.3 ns	80***
Leaf biomass	66.4***	2.4* (+)	2.4 ns	67***
Leaf mass fraction (LMF)	76.1***	0.1 ns	1.5 ns	74***
Leaf dry matter content (LDMC)	63.6***	6.3*** (-)	3.1 ns	79***
Specific leaf area (SLA)	73.2***	5.6*** (+)	2.3 ns	73***
Leaf relative water content (LRWC)	41***	9.9***(+)	3.7 ns	45***
Fruit biomass	54.7***	4.7** (+)	1.9 ns	54***
Fruit mass fraction (FMF)	68.3***	3.2* (+)	3.0 ns	69***

The proportion of the explained variance (SSx/SStotal × 100; *SS* sum of squares) and the level of significance (*ns* not significant; * $P<0.05$; ** $P<0.01$; and *** $P<0.001$) for each factor and their interactions are indicated. R^2 is the percentage of total variance explained by the model. (+) or (−) means that the biochar increased or reduced the value of the variable, respectively.

The addition of BC significantly increased plant height (from 47 to 50 cm), aboveground dry biomass (from 5.4 to 6.9 g; Fig. 2a), and stem (from 2.4 to 3 g), leaf (from 2.1 to 2.7 g), and whole plant biomass (from 7.5 to 9.1 g) (Table 2). However, BC did not affect LMF or SMF (Table 2). With respect to the leaf functional traits, BC increased SLA (from 22.9 to 25.1 $m^2\ kg^{-1}$; Fig. 2b) and LRWC (from 0.89 to 0.93 $g\ g^{-1}$; Fig. 2d), and reduced LDMC (from 0.22 to 0.19 $g\ g^{-1}$; Fig 2c). Furthermore, BC significantly increased fruit biomass (from 0.8 to 1.3 g $plant^{-1}$; Fig. 2e) and biomass allocation to fruit (FMF; from 0.1 to 0.15 $g\ g^{-1}$; Fig 2f).

Summary of the effects of biochar

As a summary, we calculated the mean BC effect on the most important soil and plant variables (see *Eq.1*). In general, BC had a stronger effect on soil chemical than on soil physical characteristics (Fig. 3a). There was an increase in the K (to more than four times its initial value), P, and Mn contents and in EC (to about twice the initial value, Fig. 3a). At the plant level, BC affected mostly fruit production (more than doubling it) and FMF, compared with the control treatment (Fig. 3b).

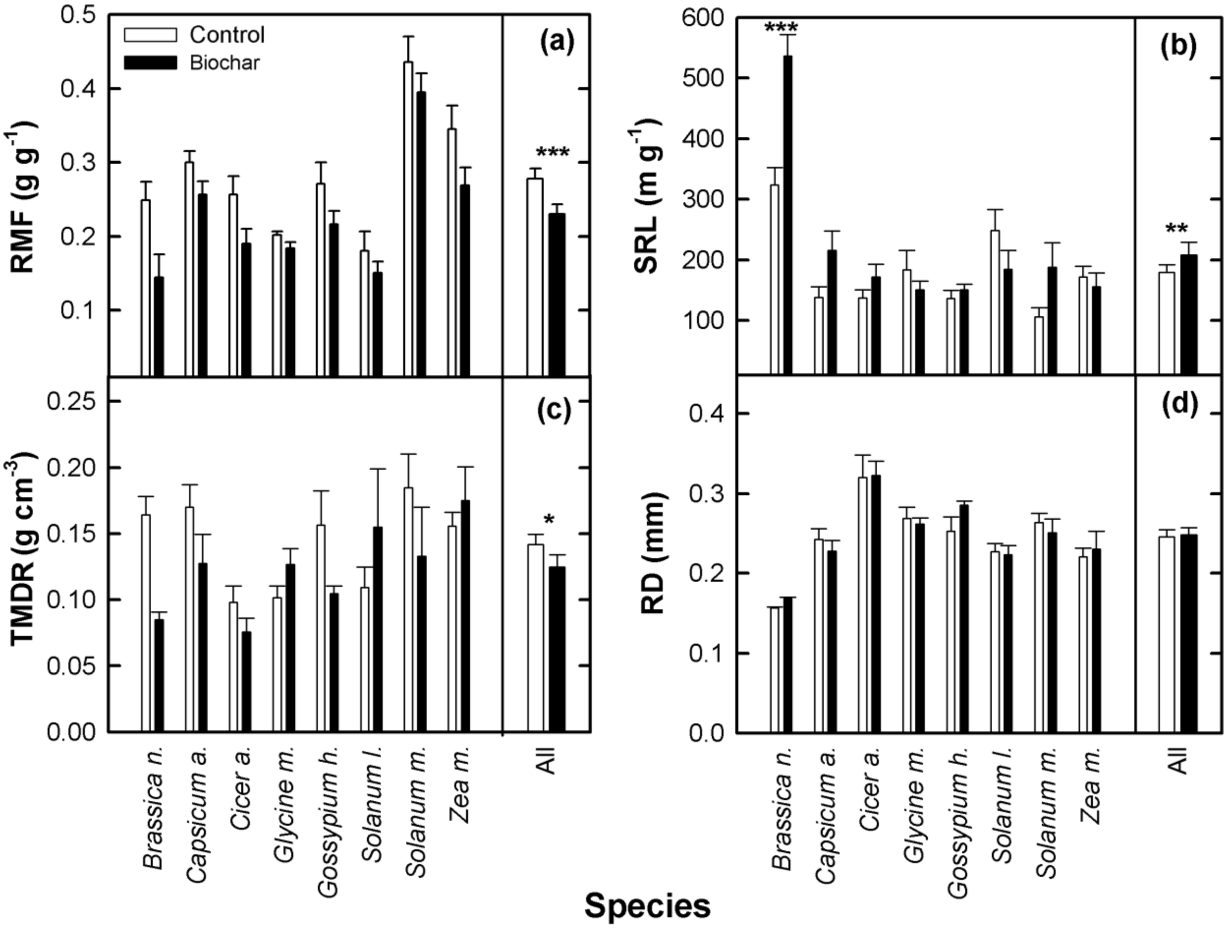

Fig. 1 Mean values ± SE (n= 4) of the root traits for each species and the average for all species, for the two treatments (Control and Biochar). **(a)** Root mass fraction (RMF); **(b)** specific root length (SRL); **(c)** root tissue density (TMDR); and **(d)** root diameter (RD). Species were ordered alphabetically. * *P*<0.05; ** *P*<0.01; and *** *P*<0.001.

The positive effects of BC on fruit production and FMF were associated with an increase in SRL (Fig. 4a, b). Therefore, those species that showed a higher increase in SRL after BC addition also had a higher increase in fruit production (*Brassica n*., *Capsicum a*., *Cicer a*., and *Solanum m*.; Fig. 4a). The increase in SRL after BC addition was due to a decrease in TMDR, not RD (Supplementary Fig. S2).

Discussion

Our results indicate that changes in soil properties (reduction in bulk density and increase in soil water content and nutrient availability) induced by BC addition initiated various responses in the roots that affected the development and production of the different plant species.

Root traits were affected by changes in soil properties after biochar addition

The reduction in bulk density and the increase in soil water content after BC addition were a direct consequence of the nature of the BC, characterized by a low density and a high porosity (Brewer et al. 2009; Verheijen et al. 2010; Abel et al. 2013). Our results also show that BC enhanced the soil nutrient status, increasing the availability of soil nutrients, especially K, P, and Mn (Fig. 3a, Table 1). These increases correspond to the high levels of these nutrients in the BC. Numerous studies have shown that the nutrients contained in BC, such as K, Ca, and P, may be available to plants (Major et al. 2010; Tammeorg et al. 2014). The increased soil pH can be attributed to the alkaline pH of BC, since pyrolysis leads to an accumulation of alkaline substances in BC, which

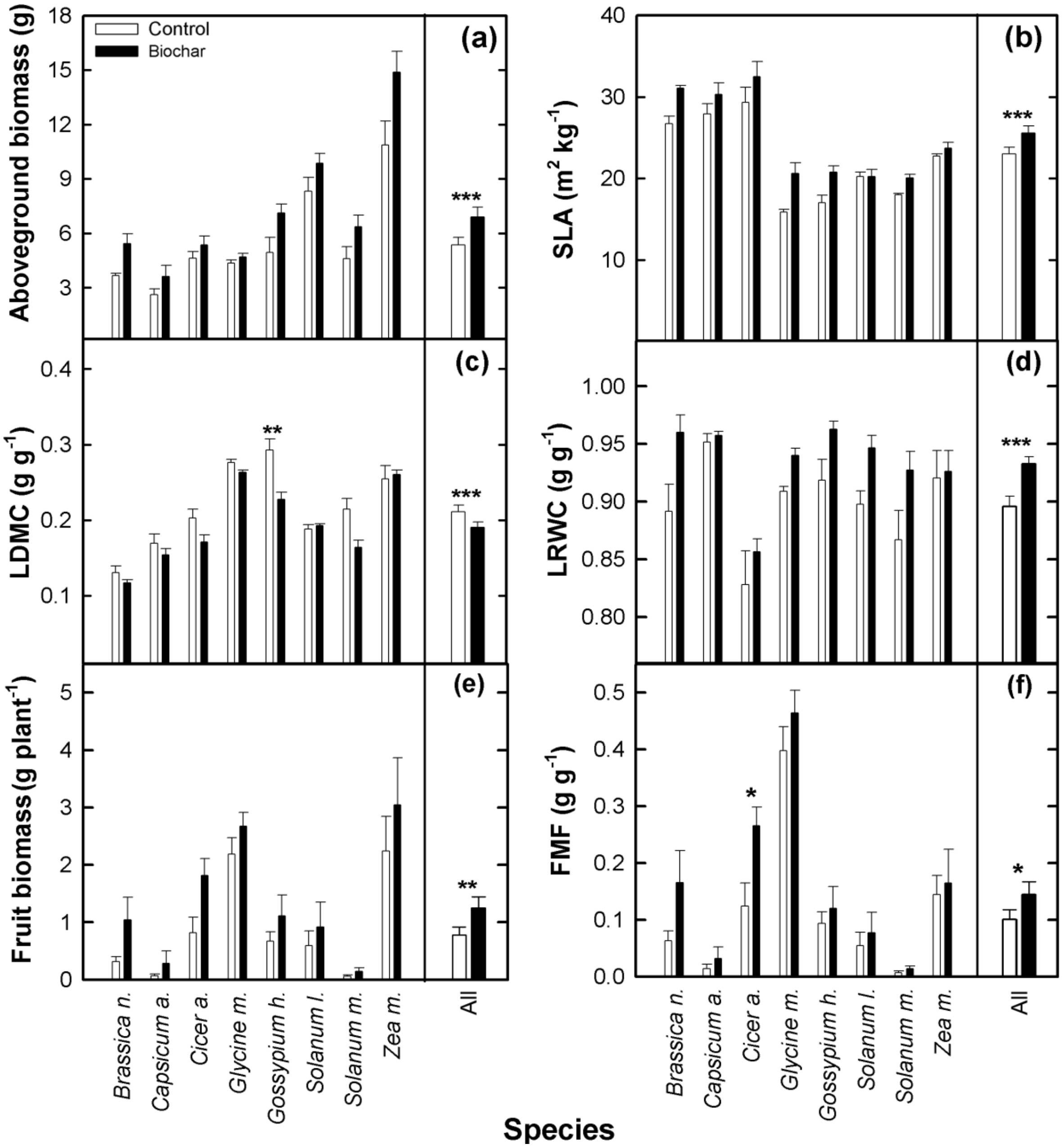

Fig. 2 Mean values ± SE (n= 6) of the aboveground plant variables and traits for each species and the average for all species, for the two treatments (Control and Biochar). (a) Aboveground biomass; (b) specific leaf area (SLA); (c) leaf dry matter content (LDMC); (d) leaf relative water content (LRWC); (e) fruit biomass; and (f) fruit mass fraction (FMF, with respect to total plant biomass). Species were ordered alphabetically. * $P<0.05$; ** $P<0.01$; and *** $P<0.001$.

gives it a strong liming effect (Van Zwieten et al. 2010; Gaskin et al. 2010). Moreover, increased soil EC is a common response to the addition of BC, since BC often has a high soluble salt content (Singh et al. 2010; Fellet et al. 2011). In our study, BC addition doubled soil EC value, so increased soil salinity must be considered when BC is applied at high rates.

Roots can be affected by the above changes in soil properties. Addition of BC reduced the biomass allocation to roots (RMF); however, this decline was due to the increase in biomass allocation to the aboveground plant parts and not to the reduction of root biomass (Table 2). This response has been described for plants growing with a high availability of resources (Müller et al. 2000; Shipley and Meziane 2002). The increased availability of water and nutrients after BC addition could reduce the

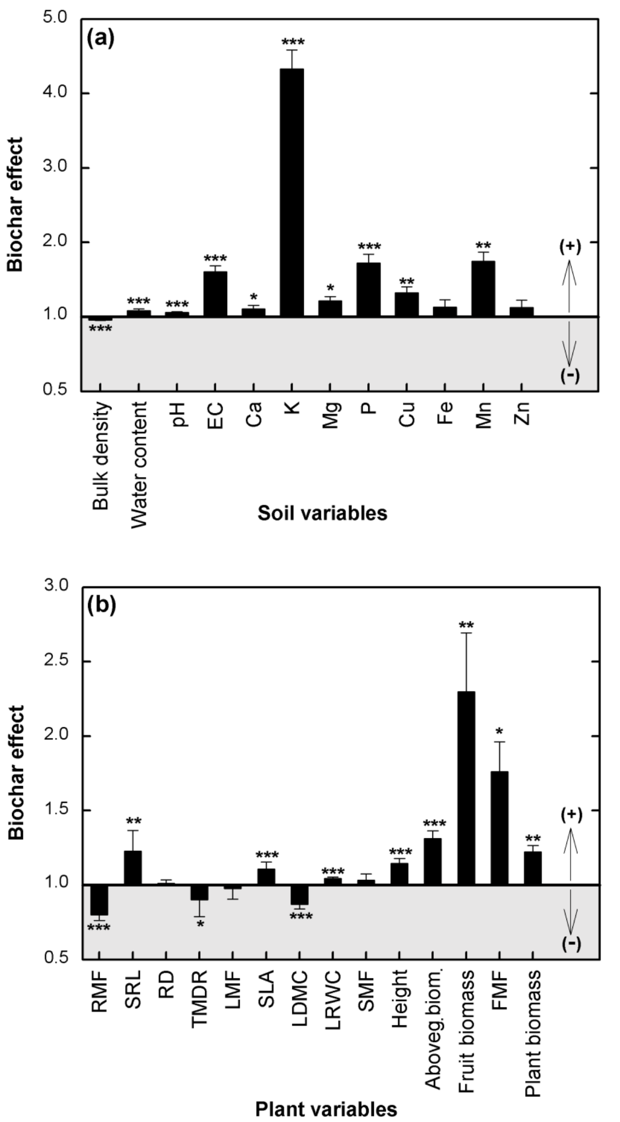

Fig. 3 Summary of the effect of biochar on **(a)** soil variables (*EC* electrical conductivity) and **(b)** plant variables and traits (*RMF* root mass fraction, *SRL* specific root length, *RD* root diameter, *TMDR* root tissue mass density, *LMF* leaf mass fraction, *SLA* specific leaf area, *LDMC* leaf dry matter content, *LRWC* leaf relative water content, *SMF* stem mass fraction, *FMF* fruit mass fraction). The biochar effect is calculated as the ratio of the mean value of a variable under the biochar treatment to its mean value under the control treatment. Biochar effects <1 are displayed in gray. The levels of significance (* P <0.05, ** P <0.01, and *** P <0.001) are indicated.

need to invest biomass in the production of roots, it being allocated instead to the development of other organs, such as stems, leaves, and fruits (Ryser and Lamber 1995; Poorter et al. 2012).

In general, BC addition increased the SRL, although it was reduced (non-significantly) in some species. This variability in the SRL response may be due to the fact that SRL depends on TMDR and/or RD (Ostonen et al. 2007; Olmo et al. 2014b). In fact, we found that BC reduced TMDR in the same species that showed an increase in SRL (Fig. 1b, c). Also, the increase in SRL after BC addition was due to the decrease in TMDR (Supplementary Fig. S2). TMDR is the ratio between root dry mass and volume, and its increase may enable a fast growth rate of roots and rapid resource acquisition, as the plant can rapidly expand the root system with a low investment of dry matter (Ryser and Lambers 1995; Wahl and Ryser 2000). In previous studies, Olmo et al. (2014a, 2015) found an increase in SRL with BC addition in a wheat crop, under both pot and field conditions. An increase in SRL means an increased root length per unit of root biomass, hence increasing the volume of soil explored per unit of root biomass (Ryser 2006; Lambers et al. 2006). Generally, SRL is related to the efficiency of resource uptake by the roots (Eissenstat 1992; Eissenstat et al. 2000) and its increase is associated with a deficient nutrient condition (Ostonen et al. 2007; Lopez-Iglesias et al. 2014). Our results agrees well with those of Olmo et al. (2015), who found that BC exerted a positive effect on some soil nutrients, while the availability of other (Mn, ammonium and nitrate) was decreased by the BC addition, which was related to an increase in the root proliferation (an SRL increase). The increase in SRL after BC addition to soil seems to be a direct result of the root attraction by BC, which maximizes biochar-root interactions and root access to the resources present in the biochar.

The increase in SRL and in nutrient acquisition efficiency could explain the reduction in RMF (the relative inversion in root biomass) in plants grown in soil treated with BC (Table 2). However, our results also suggest that the effects of BC on root morphology depended greatly on the species, although there was a general increase in the proliferation of fine roots. Therefore, the mechanisms by which BC modifies the root morphology are not entirely clear. These results confirm our first two hypotheses, since BC addition (i) improved soil characteristics, reducing soil bulk density and increasing soil water content and nutrient status, and (ii) modified root morphology, favoring fine root proliferation, and increasing SRL.

Biochar addition promoted plant growth and fruit production

The BC-treated plants showed greater development of their aerial parts, as evidenced by the increase in aboveground biomass (Table 2). In addition to the increase in leaf biomass with BC addition, there was also

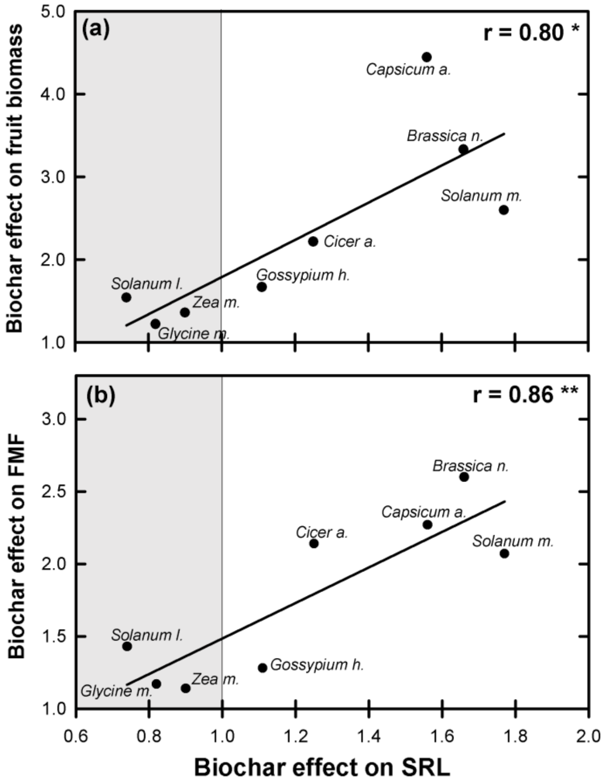

Fig. 4 Relationships between the effect of biochar on specific root length (SRL) and **(a)** the effect of biochar on the fruit biomass or **(b)** the effect of biochar on the fruit mass fraction (FMF). Species codes are indicated. The biochar effect on each variable is calculated as the ratio of the mean value of a variable under the biochar treatment to its mean value under the control treatment. Biochar effects <1 are displayed in gray. Pearson correlation coefficients and significance levels are given (* *P*<0.05 and ** *P* <0.01).

an increase in SLA (Table 2). This indicates an increase in leaf area and therefore a greater area for light absorption by the plant, which could promote plant growth in favorable conditions (Poorter and Evans 1998; Reich et al. 1998). The SLA is an important morphological trait, being a good predictor of plant productivity (Lambers and Poorter 1992; Marañon and Grubb 1993; Garnier and Laurent 1994; Poorter and de Jong 1999). The BC-treated plants had a higher LRWC and lower LDMC than control plants (Fig. 2c, d), which indicates that their hydric status was better, despite having an increased evaporative surface (an increase in SLA and leaf biomass). The increase in soil water content after BC addition and an extensive root system could explain this improvement in plant water status.

Together with the beneficial effects of BC on plant growth, our results also show an increase in fruit production, with an average increase of 59% (the mean value for all species; Fig. 3b). This yield increase is greater than that found by Jeffery et al. (2011) and Liu et al. (2013), who reported increases of about 10% after BC addition. However, these reviews also highlight a wide variation in the response of species. The addition, in the present study, of a fertilizer during the reproductive stage could also have improved markedly the yield, since BC can improve the efficiency of the fertilizers added (Chan et al. 2007; Alburquerque et al. 2013; Zheng et al. 2013). Our results show that the addition of BC had a positive effect on growth and fruit production, confirming our third hypothesis, namely that, the morphological adjustments induced by BC are related to the increase in fruit production. The positive relationships between the BC effect on SRL and the BC effect on both fruit biomass and FMF (Fig. 4) show that the species that exhibited the greatest increases in SRL in the presence of BC were the ones that showed the greatest increases in fruit yield. To generalize our findings it would be necessary to deepen the study of the responses of roots to BC addition, under different study conditions (different types of biochar and different soil types, climates, and species), before adopting BC globally as a soil amendment in agriculture.

Conclusions

Our results show that the addition of biochar produced from olive-tree prunings had a strong influence on soil properties, which could explain its effects on root traits. In general, all the species tested responded to biochar by reducing their root mass fraction and modifying their root morphology (manifested by an increase in specific root length and a decrease in root tissue density). Plants grown in soil treated with biochar showed greater development of their aerial parts (increased aboveground biomass, leaf specific area, and leaf relative water content) and enhanced fruit production. The effects of biochar on root morphology could improve plant growth and explain its positive effects on fruit production. Changes in the specific root length may therefore serve as an important indicator of soil changes induced by biochar and its study can contribute to a better understanding of the effects of biochar on plant yield.

Acknowledgements

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Compliance with ethical standards. This article does not contain any studies with human or animal subjects.
Conflict of interest: The authors declare that they do not have conflicts of interest.

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Supplementary Information

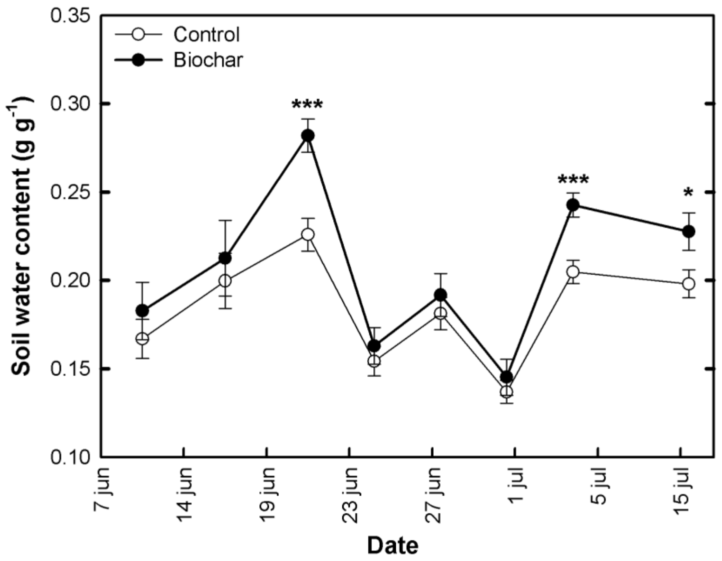

Fig. S1 Mean values ± SE (n= 16) of soil water content for the control and biochar treatments during the experiment. * *P*<0.05 and *** *P*<0.001.

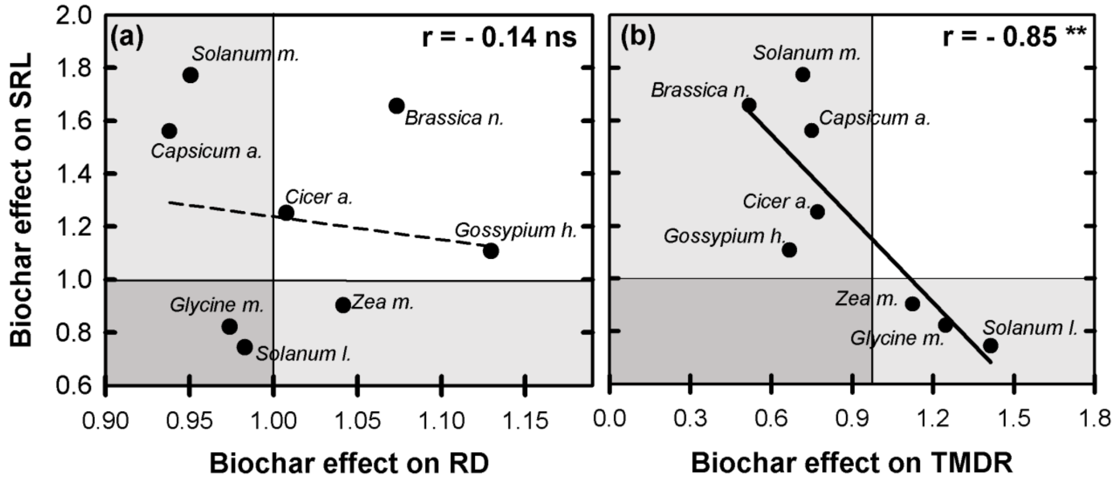

Fig. S2 (a) Relationship between the effect of biochar on specific root length (SRL) and **(a)** the effect of biochar on root diameter (RD); or **(b)** the biochar effect on root tissue density (TMDR). Species codes are indicated. The biochar effect on each variable is calculated as the ratio of the mean value of a variable under the biochar treatment to its mean value under the control treatment. Biochar effects <1 are displayed in gray. Pearson correlation coefficients and significance levels are given (*ns* not significant, ** *P*<0.01)

Appendix S1

Analytical methods

The following methods were used for soil characterization. The pH was determined in a 1:2.5 (w/v) soil/water extract with a pH meter with a glass electrode, and the electrical conductivity in a 1:5 (w/v) soil/water extract with a conductivimeter. Available P was extracted with 0.5 M $NaHCO_3$ (1:10, w/v) for 30 min and measured by the colorimetric method ("Olsen P"; Olsen and Sommers 1982). Available K, Ca, and Mg were extracted with 1 N NH_4OAc, while available Fe, Mn, Cu, and Zn were extracted with 0.05 M EDTA: all were determined by atomic absorption spectrophotometry. The calcium carbonate equivalent was determined according to the method of van Wesemael (1955) and soil organic C was determined according to the method of Walkley and Black (1934).

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Capítulo 4. Wheat growth and yield responses to biochar addition under Mediterranean climate conditions

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SPECIAL ISSUE

Wheat growth and yield responses to biochar addition under Mediterranean climate conditions

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Abstract The effects of the addition of a slow pyrolysis biochar (produced from olive-tree prunings) to a vertisol were studied in a field experiment during one wheat (*Triticum durum* L.) growing season. The biochar addition did not significantly affect soil parameters such as pH, dissolved organic C and N, ammonium, nitrate or microbial biomass N. By contrast, biochar addition decreased soil compaction and increased the soil water-retention capacity and nutrient content (total N and the available contents of P, K, Mg, Cu and Zn). These favourable changes led to an increase in fine root proliferation (increasing specific root length and reducing root tissue density) and promoted crop development. As a result, the plants in biochar-treated plots showed higher relative growth and net assimilation rates, aboveground biomass and yield than those in control plots. Neither grain quality nor nutrient content were significantly affected by biochar addition. Our results suggest that the use of biochar as a soil amendment in agricultural soils can improve soil physical properties and increase fertility, favouring crop development under semiarid Mediterranean conditions.

Keywords Soil fertility · Plant growth · Relative growth rate · Root traits · Specific leaf area · Specific root length

Electronic supplementary material The online version of this article (doi:10.1007/s00374-014-0959-y) contains supplementary material, which is available to authorized users.

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Introduction

The atmospheric CO_2 concentration continues to rise due to human activities, resulting in global warming with negative consequences for human health, food production and biodiversity (IPCC 2013). The implementation of climate change mitigation strategies based on soil C sequestration and reduction in greenhouse gas emissions is necessary to reduce the environmental impact and favour the sustainability of agricultural production systems. On the other hand, reduction of soil fertility and productivity due to organic matter loss is one of the most important soil problems for agronomic systems in areas with a Mediterranean-type climate (EEA 2010). Under this scenario, the use of biochar (BC) can be an effective tool for sustainable agriculture in the long term, increasing soil C sequestration (C abatement strategy), fertility and productivity (soil quality) and reducing greenhouse gas emissions (Jeffery et al. 2014). The European Biochar Certificate (EBC 2013) defined BC as 'a charcoal-like substance that is pyrolysed from sustainable obtained biomass under controlled conditions and is used for any purpose which does not involve its rapid mineralisation to CO_2'.

In order to offset the cost of BC production, different residual biomasses coming from agriculture, food and forestry wastes can be used as feedstock. Olive-tree prunings were used in the present study, because they are produced in large quantities in the Mediterranean region (about 7 million tons of olive-tree prunings are produced yearly in Spain; Toledano et al. 2012). Although several studies have dealt with the use

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of olive-tree prunings to produce power, heat, chemical products and biofuels, the disposal of this residue represents a problem due to the lack of cost-effective industrial applications (Spinelli and Picchi 2010; Toledano et al. 2012). Nowadays, olive-tree prunings are usually crushed and incorporated into the soil or burnt in the field to prevent the propagation of plant diseases. This causes economic cost and environmental concerns, being necessary to assess a proper disposal for olive-tree prunings. Previous pot experiments under controlled growth chamber and greenhouse conditions have shown that crops respond positively to additions of olive-tree pruning BC (Alburquerque et al. 2013, 2014). However, these results need to be reproduced under field conditions in order to demonstrate the effectiveness of BC as soil amendment for enhancing the quality of agricultural soils.

Recent studies have reviewed in detail the impact of BC addition on crop yield, which is the critical factor to assess BC as a valuable resource for agriculture (Crane-Droesch et al. 2013; Mukherjee and Lal 2014). These studies have underlined that the adoption of BC-based practices in agriculture is mainly limited by the uncertainty of the obtained results since they are highly dependent on BC type, application rate, soil properties and environmental conditions. To date, only a few studies dealing with field trials are available. For example, positive effects of BC addition up to 30 % on wheat (Baronti et al. 2010; Vaccari et al. 2011) and maize (Baronti et al. 2010) yield have been reported, and also negative/non-relevant effects on maize yield (Gaskin et al. 2010), wheat yield (Tammeorg et al. 2014) or vine growth and grape quality parameters (Schmidt et al. 2014) have been shown. Rogovska et al. (2014) noted a significant increase in maize grain and biomass yields (11–55 %) in the first year after BC application, but no clear effects were observed in the second year. Therefore, there is further need for research focusing on field studies under site-specific conditions (available feedstocks, climate conditions, type of soil and crop, etc.) to elucidate mechanisms of BC action. Several mechanisms have been proposed to justify the benefits of BC as soil amendment for agriculture, such as liming effect in acidic soils (Atkinson et al. 2010; Laird et al. 2010; Vaccari et al. 2011; Yuan and Xu 2011); increases in soil nutrient status (Glaser et al. 2002; Laird et al. 2010; Xu et al. 2013; Tammeorg et al. 2014), water-holding capacity (Brockhoff et al. 2010; Laird et al. 2010; Baronti et al. 2014; Rogovska et al. 2014) and cation exchange capacity (Glaser et al. 2002; Liang et al. 2006; Laird et al. 2010); enhancement of soil biological activity (Lehmann et al. 2011); and immobilisation of contaminants (Qian et al. 2013; Rogovska et al. 2014).

Most of the studies of BC effects on crop growth and yield are focused on total biomass analysis. However, to understand how crop growth and yield can be affected by BC addition, it is necessary to evaluate its impact on key morphological and physiological plant traits such as belowground plant traits (specific root length (SRL), root tissue mass density (TMDr) and root diameter (RD)) and aboveground plant traits (specific leaf area (SLA), net assimilation rate (NAR), leaf area ratio (LAR) and dry mater content (DMC)) (Ostonen et al. 2007; Lambers et al. 2008; see Table 1 for more details). As far as we know, there is no study about the effects of BC on these plant growth variables under field conditions, being the main novelty of this research.

The objectives of this study were to evaluate changes in soil parameters (pH, electrical conductivity, organic C, total N, dissolved organic C and N, microbial biomass N, available nutrients and compaction) caused by BC addition; to determine how these changes affect below and aboveground plant variables and traits; and to explain how plant trait changes may determine the plant growth and crop yield. Three hypotheses were tested: (i) BC addition alters soil physico-chemical properties, acting positively on soil water and nutrient status and reducing soil compaction; (ii) these BC-induced effects favour root proliferation; and (iii) as a result, plants in BC-treated soil show a higher growth rate and crop yield.

Materials and methods

Experimental design

The experiment was conducted during the 2012/2013 season in experimental plots of the 'Encineño' farm, located near Santa Cruz (Córdoba, southern Spain; 37° 46′ 54″ N, 4° 35′ 33″ W, 256 m above sea level), using durum wheat (*Triticum durum* L.) 'Carpio'. The average temperature was 13 °C (mean of 7 months), with a maximum temperature of 43 °C in June and an average relative humidity of 69 % (data logger PCE-HT71, PCE-Instruments, Tobarra, Spain) (Fig. 1a). The total rainfall for the agronomic year was 769 mm. The soil, classified as a Vertic Calcixerert (USDA Soil Taxonomy), had a clay texture (22 % sand, 27 % silt and 51 % clay), a pH of 8.2 (1:2.5 soil/water), 327 g kg^{-1} calcium carbonate equivalent, an electrical conductivity (EC) of 0.15 dS m^{-1} (1:5 soil/water), 8.26 g kg^{-1} organic C (C_{org}), 1.07 g kg^{-1} total N, 12.9 mg kg^{-1} Olsen P and a cation exchange capacity of 51.7 $cmol_+ kg^{-1}$.

Olive-tree prunings were provided by 'Valoriza Energía Puente Genil' (Córdoba) and pyrolysed in a pilot plant at 450 °C (slow pyrolysis), by the University of León (Natural Resources Institute, Spain). For the chemical characterisation, the BC was ground in a stainless steel mill to <2 mm and showed the following characteristics: pH of 9.45 and EC of 1.36 dS m^{-1} (1:10 BC/water); cation exchange capacity of 29.6 $cmol_+ kg^{-1}$, 642.2 g kg^{-1} C_{org} and 12.1 g kg^{-1} total N. Phytotoxicity tests using cress (*Lepidium sativum* L.) and lettuce (*Lactuca sativa* L.) did not show inhibitory effects of BC (germination index >70 %). The BC showed molar H/C_{org} of 0.5, molar O/C_{org} of 0.1 and 9.1 mg kg^{-1} polycyclic

Table 1 The studied variables with their abbreviations, units, descriptions and functional roles

	Variable	Abbreviation	Units	Description	Functional role
Soil	Water content		$g\ g^{-1}$	Water per unit of soil mass	Water available to plants
	Compaction		MPa	Resistance to penetration	Mechanical resistance of soil to root growth
Growth	Relative growth rate	RGR	$mg\ g^{-1}\ day^{-1}$	Plant biomass increase per unit of biomass and time	Integrates the physiological and morphological responses to the environment, being the product of the net assimilation rate and leaf area ratio
	Net assimilation rate	NAR	$g\ m^{-2}\ day^{-1}$	Plant biomass increase per leaf area and time	Physiological component of growth. Net balance between gain (photosynthesis) and losses (respiration) of the plant
	Leaf area ratio	LAR	$m^2\ kg^{-1}$	Ratio of leaf area to plant biomass	Morphological component of growth. Quantifies the inve tment in photosynthetic organs
Leaf	Specific leaf area	SLA	$m^2\ kg^{-1}$	Ratio of leaf area to leaf biomass	Leaf morphological traits related to the acquisitive strategie
Spike	Dry matter content	DMC_{spike}	(%)	Ratio of spike dry mass to fresh mass	Related to the density of the wheat spike and the degree of grain filling
Root	Specific root length	SRL	$m\ g^{-1}$	Ratio of root length to root biomass	Root morphological trait related to the acquisitive strategy. In icate root proliferation and potential resource uptake
	Root tissue density	TMDr	$g\ cm^{-3}$	Ratio of root mass to root volume	Root structure and resistance of roots to tension
	Root diameter	RD	mm	Mean root diameter	Penetration ability

aromatic hydrocarbons (PAHs, sum of the EPA's 16 priority pollutants; toluene extract). All these values fulfilled the quality requirements suggested by The European Biochar Certificate (EBC 2013). Details of the analytical methods for BC characterisation can be found in Alburquerque et al. (2013, 2014).

The experimental design consisted of a randomised complete block with plots of 15 m^2 (2.5×6 m), considering two treatments: control (without BC addition) and soil treated with BC. There were four plots per treatment, resulting in a total area of 60 m^2 for each treatment. Before the wheat was sown, the BC (as received, unmilled) was added manually to the plots and immediately incorporated into the soil (to a depth of about 20 cm), to ensure uniform distribution and avoid wind losses. The same type of soil work was applied to all plots (treated and non-treated with BC). The BC application rate was 4 kg m^{-2} (40 Mg ha^{-1}, equivalent to approximately 2 % (*w/w*) on a dry weight basis), which was selected based on previous experiments and commonly used in field experiments (Vaccari et al. 2011; Rogovska et al. 2014; Tammeorg et al. 2014). The plots were separated from each other by 3 m.

Wheat was sown on 12 December 2012, in 20-cm-wide rows (approximately 760 seeds/m^2) and grown for 7 months. Crop management followed the standard agronomic practices used in the area regarding soil preparation, fertilisation (110 kg ha^{-1} top dressing of urea; 5 December) and herbicide (500 g ha^{-1} of a mixture of mesosulfuron-methyl (3 %)+ iodosulfuron-methyl (0.6 %); 20 March) and fungicide (0.4 L ha^{-1} pyraclostrobin (20 %) and 0.8 L ha^{-1} epoxiconazole (12.5 %); 9 April) treatments.

Plant samplings

During the wheat growing season, three plant samplings were carried out.

First sampling It was conducted on 17 January 2013 (36 days after sowing (DAS)) to establish the initial crop conditions. One area of 25×25 cm/plot was randomly selected to sample wheat plants, avoiding edge effects. Roots were also sampled in the selected area to 10-cm depth, where most of the roots were at this early stage of development. In the laboratory, the number of plants was counted and ten individual plants were randomly selected in order to obtain data per individual plant (growth analysis). The rest of the plants in the sample area were harvested and oven-dried (70 °C for 72 h), then the weights of the leaves, stems and roots were determined. For the ten selected plants, the leaves were scanned and leaf area was measured with the software Image Pro v4.5 (Media Cybernetics, Atlanta, GA). The roots were gently washed from the soil with tap water, taking care to avoid the loss of roots. The roots were scanned at a resolution of 420 dpi and root length, volume and diameter were calculated using WinRHIZO Pro 2004 (Regent

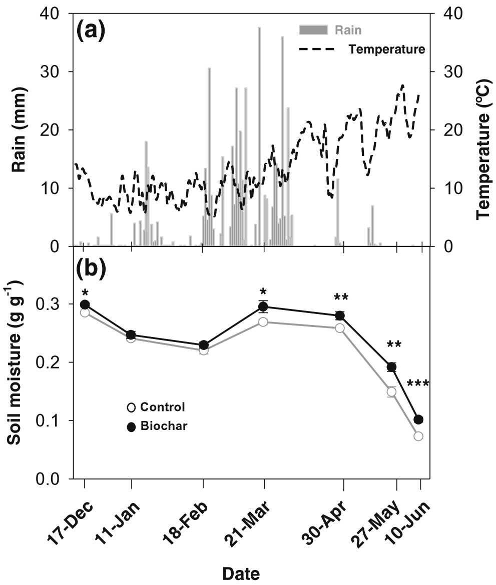

Fig. 1 Average air temperature and rain (**a**) and soil moisture (**b**) for control and biochar treatments (mean value±SE, *n*=4). **P*<0.05; ***P*<0.01; ****P*<0.001

Instruments Inc.) (Himmelbauer et al. 2004). Finally, the roots were oven-dried, at 70 °C for 72 h and weighed.

Second sampling It was conducted on 15 April 2013 (124 DAS) in order to evaluate the effects of BC on plant growth and key functional traits. The crop was at the post-anthesis stage and the grain was at the early dough stage (Zadoks et al. 1974). The methodology was similar to that of the first sampling, with sampling of one randomly selected area of 25×25 cm/plot. The plant biomass (on both fresh and dry weight bases) was separated into the spike, flag leaf, leaves, stems and roots. The leaf area was determined for both the flag leaf and other leaves. Soil cores, to sample the roots, were taken with a cylindrical metal tube (8.5-cm diameter and 30-cm long) at the same site from which the aerial part had been removed previously (25×25 cm area). Two root samplings were conducted in each square of 625 cm^2 (one within the plant row and one between two rows). The root sampling was performed every 10 cm to a depth of 30 cm. In the laboratory, roots were carefully separated from the soil, cleaned of soil particles and scanned, following the same methodology as in the first sampling.

Third sampling and harvesting To evaluate the effect of BC on the final wheat biomass and yield, a third sampling was performed on 10 June 2013 (187 DAS), following the same methodology as the previous samplings except that in this case two areas of 50×50 cm/plot were randomly selected. In the laboratory, the aerial part was separated into the leaves, stems, spikes and grain to obtain the dry weight (70 °C for 72 h). Finally, the wheat was harvested with a 1.8-m-wide mini-harvester (12 June 2013). For each plot, 10.8 m^2 (1.8×6 m) were harvested to obtain the grain yield.

Plant variables and data analysis

A summary of the variables studied for growth and yield analysis, with their description and functional role, can be found in Table 1. It included the relative growth rate (RGR) and its components: LAR and NAR, as RGR=LAR×NAR. The RGR, LAR and NAR were calculated according to Hunt et al. (2002). In addition, functional traits related to leaf, spike and root were measured: SLA, dry matter content of the spike (DMC_{spike}), SRL, TMDr and RD. See Table 1 for a complete description and calculation of variables.

Soil samplings

During the experiment, the soil gravimetric water content and soil penetration resistance were measured monthly. For soil gravimetric water content, two soil samples (depth, 20 cm) were taken per plot, and it was calculated as ((wet weight−dry weight) / dry weight)×100. For soil penetration resistance, four measurements per plot were taken with a penetrologger (Eijkelkamp, Giesbeek, The Netherlands) using a cylindrical probe of 3.3 cm^2 and 30° cone angle, taking force measurements every centimetre to a total depth of 30 cm.

At the end of the experiment, soil samples (depth of about 20 cm) were taken at ten different, random sites in each plot and combined to obtain a representative sample per plot. Each sample was divided into two fractions: one was immediately sieved to <2 mm and stored without drying at <4 °C for measuring dissolved organic C, dissolved N and microbial biomass N; the other fraction was air-dried. One aliquot of the air-dried soil sample was sieved (<2 mm) for physico-chemical and chemical analyses.

Analytical methods

Air-dried and sieved (2 mm) soil was analysed for pH, EC, particle-size distribution, $CaCO_3$, organic C, total N and available nutrient contents. The pH was determined in a 1:2.5 (*w/v*) soil/water extract (pH meter with glass electrode) and EC in a 1:5 (*w/v*) soil/water extract using a conductivity bridge. The particle-size distribution was determined by the pipette method (Gee and Bauder 1986), the $CaCO_3$ content with a Bernard calcimeter (Duchaufour 1965) and the cation exchange capacity by extraction with 1 M NH_4OAc buffered at pH 7 (Rhoades 1982). Soil organic C was determined according to the Walkley-Black method (Walkley and Black 1934), and total N following the Kjeldahl procedure (Hesse 1971). The soil available P was extracted with 0.5 M $NaHCO_3$ (1:10, w/v) for 30 min and measured colorimetrically ('Olsen P'; Olsen and Sommers 1982). Available Mg and K were extracted with

1 N NH_4OAc and available Fe, Mn, Cu and Zn with 0.05 M EDTA; all being measured by inductively coupled plasma optical emission spectrometry (ICP-OES).

The soil contents of labile organic C and N forms included respectively, dissolved organic C (DOC), and dissolved organic N (DON) and microbial biomass N. The DOC was determined with an automatic analyser using liquid samples and DON was estimated as the difference between total N and inorganic N in K_2SO_4 extracts. Microbial biomass N was determined using the fumigation-extraction method (Brookes et al. 1985), as the difference between the total N of fumigated and unfumigated 0.5 M K_2SO_4 extracts. The extracts were digested with $K_2S_2O_8$ in an autoclave at 121 °C for 55 min and then incubated with Devarda's alloy overnight. The total N in the digested extracts as well as the NH_4^+–N and NO_3^-–N were determined by colorimetry (indophenol blue method) using a microplate reader (Sims et al. 1995).

Wheat grain quality and nutrient uptake were analysed on representative samples dried at 70 °C for 72 h, weighed and digested in nitric/perchloric acid. The resulting solution was analysed for Ca, Mg, Fe, Mn, Cu and Zn by atomic absorption spectrophotometry, for K by flame emission and for P with the molybdenum blue colour method of Murphy and Riley (1962). Grain vitrosity (%) was determined by counting the number of vitreous kernels from a total of 50 grains sliced with a Pohl grain cutter. Fourier transform near infrared (FT-NIR) spectroscopy was used to determine the moisture and protein contents, with a Foss Infratec 1241 Grain Analyzer. All physico-chemical analyses were performed in duplicate and soil microbial biomass in triplicate.

Statistical analysis

The data at each sampling date were analysed by analysis of variance (ANOVA) for a randomised complete block design. If necessary, data were transformed before statistical analysis to fulfil the assumptions of ANOVA. Regression analyses were used to determine the relationships between variables. All statistical analyses were carried out using Statistix v9 (Analytical software, USA).

Results

Effect of BC application on soil parameters

The BC addition increased significantly the soil moisture, from March to the end of the experiment (June) (Fig. 1b). In general, BC decreased soil compaction during the experiment (with an average reduction of 9 % in the 0–30 cm depth), although significant differences were only observed in a few samplings (see Table S1 in Electronic supplementary material).

At the end of the experiment (Table 2), BC addition ha increased significantly EC and contents of C_{org}, macronutrients (total N and available P, K and Mg) and micronutrients (available Cu and Zn). By contrast, no statistically significa t effects of BC addition on soil pH and contents of available Fe and Mn or labile C and N forms were found (Table 2).

Effect of BC application on belowground biomass and traits

At the first sampling (36 DAS), the BC addition did not have any significant effect on either the root biomass (Table 3) or root traits (data not shown). By contrast, at the second sampling (124 DAS), there was a nearly significant (P=0.084) increase in the SRL and a significant decrease (P=0.014) i the TMDr in the upper soil layer (10-cm depth) of BC-treate plots with respect to the control plots (Fig. 2a, b). In additio , SRL was negatively correlated to TMDr and RD (for detai s, see Electronic supplementary material Fig. S1). However, neither root biomass (Table 3) nor RD (Fig. 2c) was significantly affected by BC addition.

Effect of BC application on aboveground biomass, traits and growth

At the first sampling (36 DAS), no significant effect of BC incorporation into the soil was observed on plant density or o the aboveground biomass measured variables (Table 3). By contrast, we found that BC addition significantly (P<0.05) increased the stem, spike and aboveground plant biomass at the second sampling (124 DAS) (Table 3). In addition, significant increases in the RGR were found in BC-treated plots, vs control plots (40.4 compared with 37.8 mg g^{-1} day^{-1}; P<0.05). When RGR was broken down into morphologica (LAR) and physiological (NAR) components, RGR was positively correlated with NAR but not with LAR (Fig. 3a, b). I addition, we observed significantly higher values of NAR i plants treated with BC (1.9 compared with 1.5 g m^{-2} day^{-1}; P<0.05). Furthermore, negative correlations of SLA with RGR and NAR were found (Fig. 3c, d). The BC additio significantly affected some plant traits, decreasing both the SLA and the SLA of the flag leaf (SLA_{FL}) and increasing the dry matter content of the spike (DMC_{spike}) (Table 3).

At the third sampling (187 DAS), the BC addition i - creased all the aboveground biomass variables (leaf, stem, spike and grain) (Table 3). We also found that grain production was related to certain soil properties (Fig. 4a, b): it was negatively correlated with soil compaction and positive y correlated with soil moisture. In addition, grain productio correlated positively with SRL (at 0–10 cm depth) and negatively with TMDr (at 0–10 cm depth) (Fig. 4c, d). However, the data obtained at harvest (with the mini-harvester) did not

Table 2 Soil variables (mean value±SE, n=4) analysed for the control and biochar treatments at the end of the experiment (on a dry weight basis)

Variable	Control	Biochar	P
pH	8.26±0.02	8.24±0.02	ns
EC (dS m^{-1})	0.12±0.01	0.18±0.01	***
C_{org} (g kg^{-1})	9.40±0.29	14.80±1.38	*
DOC (mg kg^{-1})	92.5±1.2	91.4±1.5	ns
N (g kg^{-1})	1.05±0.01	1.64±0.13	*
DON (mg kg^{-1})	6.7±0.3	7.1±0.7	ns
Microbial biomass N (mg kg^{-1})	21.4±1.2	22.5±2.2	ns
NH_4^+–N (mg kg^{-1})	2.0±0.1	2.0±0.1	ns
NO_3^-–N (mg kg^{-1})	6.0±0.8	8.1±0.7	ns
Olsen-P (mg kg^{-1})	12.8±0.4	21.0±1.3	*
NH_4OAc-K (mg kg^{-1})	678.0±17.6	1,004.0±12.4	**
NH_4OAc-Mg (mg kg^{-1})	334.8±3.4	345.3±3.3	*
EDTA-Fe (mg kg^{-1})	24.9±1.1	26.0±1.0	ns
EDTA-Mn (mg kg^{-1})	26.0±0.7	27.6±1.4	ns
EDTA-Cu (mg kg^{-1})	2.2±0.1	3.8±0.3	**
EDTA-Zn (mg kg^{-1})	0.5±0.1	1.0±0.1	**

EC electrical conductivity, C_{org} organic C, *DOC* dissolved organic C, *N* total N, *DON* dissolved organic N, *ns not significant*

*P<0.05; **P<0.01; ***P<0.001

show any significant effect of BC addition on grain yield (472 ±36 vs 483±18 g m^{-2} for control and BC treatments, respectively). With respect to grain quality and nutrient content, only the Fe and Zn concentrations were slightly lower for the BC treatment (Table 4).

Discussion

Biochar effects on soil parameters

The BC-treated plots showed the highest soil moisture during the experiment, from 8 to 40 % higher than the control plots. This can be attributed to the high water retention capacity of BC, whose high porosity and high specific surface area have been described as contributing to the principal benefits of its use as a soil amendment (Glaser et al. 2002; Brockhoff et al. 2010; Makoto and Yasuyuki 2010). Although our results refer to the total amount of water in the soil, recent studies have confirmed that the water retained in BC pores is available to plants, and this can reduce plant water stress (Brockhoff et al. 2010; Baronti et al. 2014). In addition, both the low density and the high water-retention capacity of BC lead to the reductions in soil compaction (Atkinson et al. 2010; Makoto and Yasuyuki 2010), as observed in our study where BC decreased soil compaction by 9 % on average; however, the differences were not statistically significant for all samplings.

The improvement of soil physical properties caused by BC addition can play a decisive role in dry climates where water availability is the main limiting factor for agriculture. BC was a determining factor under our specific soil characteristics since vertisols generally have a good chemical fertility but poor physical properties that limit their agricultural management. They are highly compact when dry and plastic in the wet season; in addition, they possess a low permeability that confers a narrow range between deficiency and excess of water (Deckers et al. 2001).

Table 3 Plant growth variables and traits (mean value±SE, n=4) measured on a dry weight basis at the first sampling (36 days after sowing (DAS)), second sampling (124 DAS) and third sampling (187 DAS) for control and biochar treatments

Variable	1st sampling			2nd sampling			3rd sampling		
	Control	Biochar	P	Control	Biochar	P	Control	Biochar	P
Plant density (tillers m^{-2})	596±44	624±64	ns	536±25	544±57	ns	469±26	498±44	ns
Spike density (spikes m^{-2})	–	–	–	528±22	516±49	ns	455±24	472±46	ns
Leaf (g m^{-2})	29.9±2.8	30.2±2.6	ns	313±18	394±15	a	182±8	199±5	*
Stem (g m^{-2})	13.3±1.4	14.1±1.4	ns	628±36	896±37	*	370±14	441±9	**
Spike (g m^{-2})	–	–	–	132±7	209±23	*	563±18	714±30	**
Grain (g m^{-2})	–	–	–	–	–	–	442±14	561±23	**
Aboveground (g m^{-2})	43.2±4.1	44.2±3.8	ns	1,073±52	1,498±70	*	1,115±32	1,354±41	**
Root (g m^{-2})	11.6±1.2	10.8±2.7	ns	53.4±5.5	60.3±5.0	ns	–	–	–
SLA (m^2 kg^{-1})	33.8±0.9	34.4±0.7	ns	26.7±0.6	24.7±0.6	**	–	–	–
SLA_{FL} (m^2 kg^{-1})	–	–	–	26.9±0.6	25.1±0.5	*	–	–	–
DMC_{spike} (%)	–	–	–	23.8±0.5	27.3±0.4	***	–	–	–

The specific leaf area (SLA), specific leaf area of the flag leaf (SLA_{FL}) and dry matter content of the spike (DMC_{spike}) were evaluated at the individual plant level (n=40)

ns not significant (P>0.1)

[a] 0.1>P>0.05; *P<0.05; **P<0.01; ***P<0.001

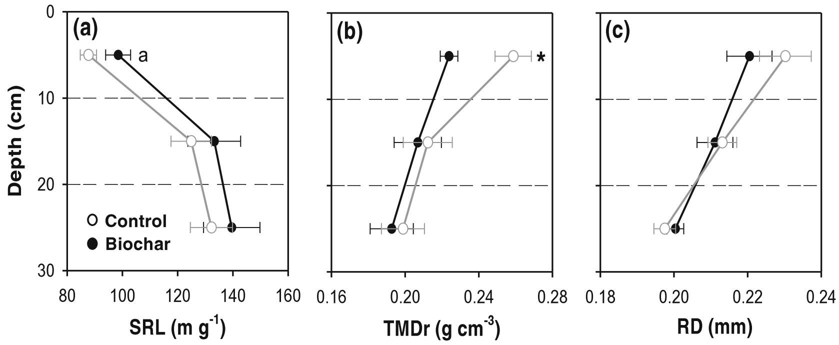

Fig. 2 **a** Specific root length (*SRL*), **b** root tissue mass density (*TMDr*) and **c** root diameter (*RD*) by depth (layer 1, 0–10 cm; layer 2, 10–20 cm; and layer 3, 20–30 cm), at the second sampling (124 DAS, mean value±SE, n=4), for control and biochar treatments; [a]0.1>P>0.05; *P<0.05

It is a well-known fact that BC addition to soil alters C and N dynamics, acting on microbial activity and hence modifying processes such as C- and N-mineralisation, biological N-fixation, nitrification and gaseous losses (Clough et al. 2013; Prayogo et al. 2014; Song et al. 2014). In addition, mineralisation and immobilisation processes greatly depend on available C and N pool to microorganisms, leading to contrasting effects after BC addition to soil (Clough et al. 2013). It is accepted that BC provides a non-easily available organic fraction to soil, which increased the total organic C and total N concentrations of soil in our experiment, but it had not a significant effect on the contents of DOC, DON, NH_4^+–N and NO_3^-–N and on microbial biomass N at the end of the experiment. The BC probably exerted a limited influence on soil C and N turnover due to its recalcitrant nature, as noted in a previous study where the same BC was applied to a loamy sand soil in a pot experiment (Alburquerque et al. 2014). On the other hand, the soil sampling at the end of the growth season coincided with high temperatures and low soil moisture contents, which can make most of soil microorganisms not active, leading to a lack of residual effects (Farrell et al. 2014).

By contrast, BC enriched the soil nutrient status with regard to available forms of P, K, Mg, Cu and Zn. These results are in agreement with previous studies (Lehmann et al. 2003; Alburquerque et al. 2013; Tammeorg et al. 2014). Changes in soil pH in response to the BC treatment have been reported (Atkinson et al. 2010; Laird et al. 2010; Yuan and Xu 2011); however, no significant change in soil pH after BC amendment occurred in this study probably because of the buffering effect of the high calcium carbonate concentration of soil (327 g kg^{-1}). The BC used in this study showed total concentrations (on a dry weight basis) of P (1.6 g kg^{-1}), K (12.4 g kg^{-1}), Ca (87.7 g kg^{-1}), Mg (4.1 g kg^{-1}), Fe

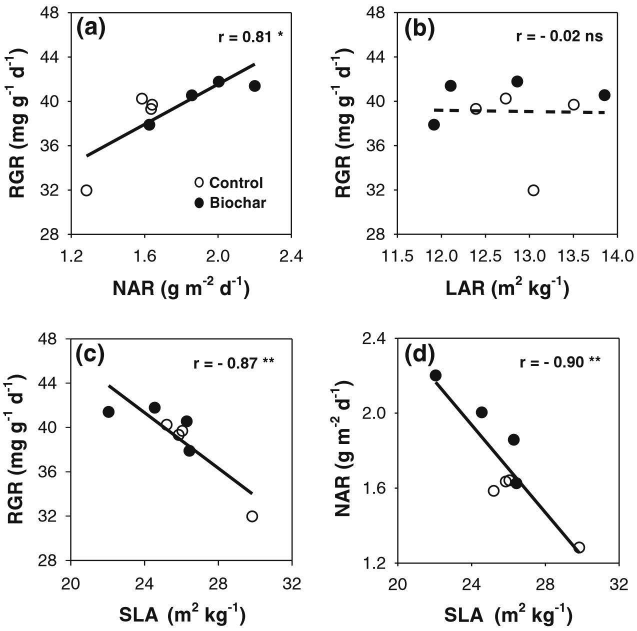

Fig. 3 Relationship between relative growth rate (*RGR*) and **a** net assimilation rate (*NAR*), **b** leaf area ratio (*LAR*) and **c** specific leaf area (*SLA*); relationship between NAR and SLA (**d**), for control and biochar treatments. Pearson correlation coefficients and significance levels are given: *ns* not significant; *P<0.05; **P<0.01

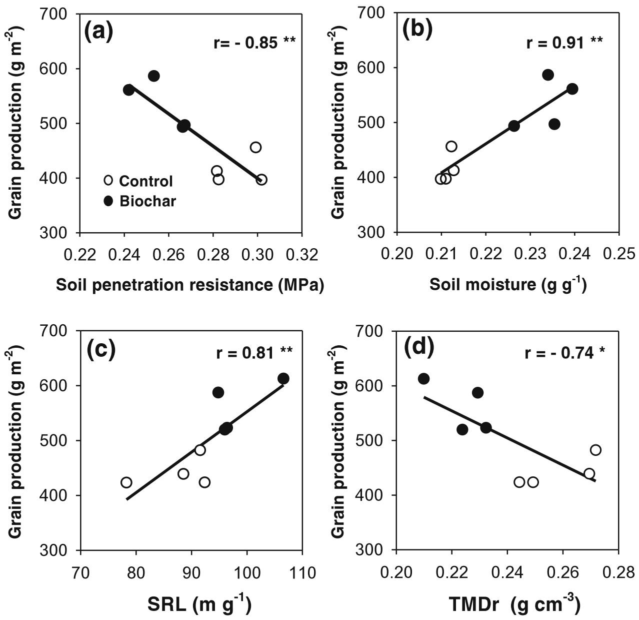

Fig. 4 Relationship between grain production and **a** mean soil penetration resistance, **b** mean soil moisture, **c** specific root length (*SRL*) and **d** root tissue density (*TMDr*), for control and biochar treatments. Pearson correlation coefficients and significance levels are given: *P<0.05; **P<0.01

Table 4 Quality parameters and nutrient content of wheat grain at harvest, for the control and biochar treatments (mean value±SE, *n*=4)

Variable	Control	Biochar	*P*
Moisture (%)	9.6±0.1	9.5±0.1	ns
Vitrosity (%)	88.0±1.7	88.5±1.3	ns
Protein (%)	10.9±0.1	11.0±0.1	ns
P (g kg^{-1})	3.97±0.16	3.90±0.12	ns
K (g kg^{-1})	4.89±0.14	4.83±0.12	ns
Ca (g kg^{-1})	5.52±0.12	5.38±0.11	ns
Mg (g kg^{-1})	1.08±0.04	0.99±0.04	ns
Fe (mg kg^{-1})	34.4±0.5	29.3±1.1	*
Mn (mg kg^{-1})	28.5±2.0	26.6±1.4	ns
Zn (mg kg^{-1})	26.2±1.4	24.4±1.1	a
Cu (mg kg^{-1})	3.5±0.2	3.2±0.2	ns

ns not significant ($P>0.1$)

[a] $0.1>P>0.05$; $*P<0.05$

(1,982 mg kg^{-1}), Mn (124 mg kg^{-1}), Zn (47 mg kg^{-1}) and Cu (136 mg kg^{-1}). Therefore, the BC must have contributed to the available forms of these nutrients. Direct effects of BC on soil fertility have been mainly related to the nutrients in mineral forms present on BC surfaces, since a low nutrient release occurs as mineralisation due to the BC recalcitrant nature (Kimetu et al. 2008; Laird et al. 2010); indirect effects have been related to the BC sorption capacity (retaining nutrients and chelating compounds) or changes in soil physico-chemical and biological properties, leading to nutrient mobilisation (Glaser et al. 2002; Laird et al. 2010; Xu et al. 2013).

The increase in soil Olsen P after BC addition is important since calcareous soils are usually deficient in available P due to the precipitation of Ca-rich phosphates and sorption processes (Solis and Torrent 1989). Several studies have underlined the fact that BC can be a valuable source of P for plant nutrition (Lehmann et al. 2003; Alburquerque et al. 2013). This effect has been related to a direct supply of P in available forms, changes in soil pH and/or an enhancement of microbial activity, leading to P mobilisation processes (Atkinson et al. 2010).

Overall, these results support our first hypothesis since BC had a positive effect on soil physico-chemical properties. The BC addition reduced soil compaction, supplied nutrients in available forms and its porous structure and high surface area retained water and nutrients, leading to a beneficial effect on soil water and nutrient status.

Biochar effects on belowground biomass and traits

The BC addition did not have a statistically significant effect on the root variables measured at the first sampling (36 DAS). However, BC-treated plants at the second sampling (124 DAS) showed a reduction in the TMDr and an increment in the SRL in the topsoil layer (0–10 cm). By contrast, root biomass and RD were not significantly affected by the BC addition. The SRL is a complex variable that depends on variations in RD and TMDr (Ostonen et al. 2007; Olmo et al. 2014). In the present study, SRL was correlated negatively with TMDr and—in a lesser degree—also with RD, which suggests that the increase in SRL was mainly a consequence of a reduction in TMDr. Therefore, reducing TMDr decreases the energetic costs of dry matter construction per unit root volume and this enables the simultaneous development of an extensive root system through fine roots. A high SRL provides an advantage for acquisition of water and nutrients (Eissenstat 1992). This suggests that the increase of fine root proliferation (increase in SRL and reduction in TMDr) by plants in BC-treated plots favoured the acquisition of the water and nutrients retained in the BC pores. Recently, Prendergast-Miller et al. (2013) evaluated the effects of BC on the roots of spring barley and found that BC-treated soils had larger rhizosphere zones than the control soil. This suggests that roots respond positively to BC particles, since root growth and development are mainly controlled by the nutrient and water availability. In our study, root responses to BC addition were mainly found in the upper soil layer (10 cm), where a high proportion of the BC was found and most of the root biomass (about 70 %) was located.

The reduction of soil compaction by BC addition could have also affected the root traits. Our results are consistent with those of Alameda and Villar (2012), who reported a reduction of SRL with increasing soil compaction, and also with those by Easson et al. (1995), who described a TMDr reduction as a response to reduced soil compaction and low tensile strength. Therefore, our results confirmed the second hypothesis and underline that BC addition to soil promoted fine root proliferation due to more favourable soil conditions (less compaction, more pore space and higher availability of water and nutrients in BC).

Biochar effects on aboveground biomass, traits and plant growth

The BC addition did not affect wheat plant density, and there was no statistical difference in the aboveground variables measured at the first sampling (36 DAS, vegetative phase), probably due to the short period of time that had elapsed since sowing and BC addition to soil. In the second sampling (124 DAS, reproductive phase), significant increases in the aboveground plant biomass variables due to BC were observed.

It is recognised that plants modify their growth with changes in environmental factors (water and nutrient availability, climatic conditions, etc.) (Chapin et al. 1987; Lambers et al. 2008). In the present study, plants of BC-treated plots showed higher RGR and NAR than plants in control plots, suggesting that BC promoted plant growth by increasing leaf photosynthetic efficiency (NAR). Increases in photosynthetic activity after BC addition have recently been related to an alleviation of water and disease stresses (Baronti et al. 2014; Wang et al. 2014). Similar to our results, several studies found that an increase in RGR was related to an increase in NAR (Villar et al. 2005; Quero et al. 2008). An increase in the leaf photosynthetic efficiency (NAR) has been also related to a reduction in the SLA (Reich et al. 1998), due to a likely increase in the amount of photosynthetic machinery per unit leaf area. Therefore, our results found a negative correlation between RGR and SLA, as did Quero et al. (2008). Rawson et al. (1987) examined this relationship in wheat and found that SLA declined in plants with more advanced crop development and higher RGR. On the other hand, the BC addition to soil also increased the spike dry matter content (DMC_{spike}). Araus et al. (1986) reported a progressive increase in spike DMC with crop development. Overall these results indicate that plants in the BC-treated soil presented a more advanced development stage than the control plants. The changes of plant growth variables, SLA, DMC_{spike} and spike biomass provoked by the BC addition before the start of the dry season are interesting under Mediterranean climate conditions because some studies have reported that greater development during the post-anthesis phase may stimulate crop yield and in this way production is less affected by heat and drought stress (Yang and Zhang 2006).

The results of the third sampling (187 DAS) seem to confirm it, as BC application also had positive effects on the aboveground and spike biomass, and on grain production, confirming data obtained in the second sampling for these variables and our third hypothesis. Thus, grain production changes induced by BC were significant (P<0.01) at the third sampling (187 DAS) but not after harvesting with the mini-harvester. This could be due to the high variability in grain production of control plots found with the mini-harvester (381 to 581 g m^{-2}). Mechanical harvesting had the advantage over manual harvesting that the harvesting area per plot was larger (10.8 vs 0.5 m^2), but the disadvantage that small variations in the harvested area might result in a considerable increase or reduction in the amount of grain harvested. Nevertheless, the data from the third sampling and harvest are not contradictory, which suggests that the BC addition did increase the crop yield. At the third sampling, we found that grain production was correlated significantly and positively with soil moisture, EC, total N, Olsen P and available K, Cu and Zn (P<0.01), and negatively with soil compaction (P<0.01), consistent with the favourable changes in soil physico-chemical properties brought about by BC addition. In addition, grain yield correlated positively with SRL and negatively with TMDr, suggesting that traits which determine root proliferation have much importance in crop yield. By contrast, BC addition did not have a great impact on grain quality or nutrient composition with only slightly lower concentrations of F and Zn in the grain of the BC-treated plots compared with the control.

Conclusions

The main innovative aspect of the present study is the int - gration of functional variables of plant growth (below and aboveground plant traits) into BC research under field experimental conditions. We demonstrated that plants responded to BC addition by increasing fine root proliferation, which favoured water and nutrient acquisition and promoted crop development and yield. Further research is needed to clarify the interaction between BC particles and roots, to assess th long-term effectiveness of BC and its economic value, to design BC with some specific characteristics for enhanci g crop yield and to evaluate the benefits of combining BC with other organic amendments (compost, digestate, manure, etc.).

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Supplementary material

Table S1 Soil penetration resistance (MPa; mean value ± SE, n=4) at different depths for the control and biochar treatments. The differences were statistically significant (P<0.05) only at days 109 (0-5 cm depth) and 149 (15-20 cm depth)

		Time after sowing (days)				
Depth (cm)	**Treatment**	**16**	**40**	**78**	**109**	**149**
0-5	Control	0.16 ± 0.01	0.19 ± 0.01	0.23 ± 0.02	0.13 ± 0.01	0.22 ± 0.05
	Biochar	0.15 ± 0.01	0.16 ± 0.01	0.24 ± 0.01	0.11 ± 0.01	0.15 ± 0.03
5-10	Control	0.20 ± 0.01	0.22 ± 0.01	0.26 ± 0.02	0.17 ± 0.02	0.45 ± 0.06
	Biochar	0.20 ± 0.01	0.21 ± 0.01	0.27 ± 0.01	0.15 ± 0.01	0.33 ± 0.04
10-15	Control	0.22 ± 0.01	0.24 ± 0.01	0.27 ± 0.02	0.20 ± 0.02	0.53 ± 0.03
	Biochar	0.21 ± 0.01	0.23 ± 0.01	0.28 ± 0.02	0.16 ± 0.01	0.41 ± 0.07
15-20	Control	0.25 ± 0.02	0.25 ± 0.01	0.32 ± 0.03	0.21 ± 0.02	0.65 ± 0.01
	Biochar	0.23 ± 0.03	0.26 ± 0.01	0.31 ± 0.01	0.19 ± 0.02	0.44 ± 0.06
20-25	Control	0.27 ± 0.03	0.30 ± 0.01	0.39 ± 0.04	0.23 ± 0.03	0.66 ± 0.04
	Biochar	0.24 ± 0.02	0.30 ± 0.02	0.35 ± 0.03	0.20 ± 0.01	0.51 ± 0.04
25-30	Control	0.31 ± 0.02	0.38 ± 0.02	0.43 ± 0.04	0.24 ± 0.02	0.60 ± 0.03
	Biochar	0.31 ± 0.04	0.35 ± 0.04	0.39 ± 0.03	0.23 ± 0.01	0.58 ± 0.02

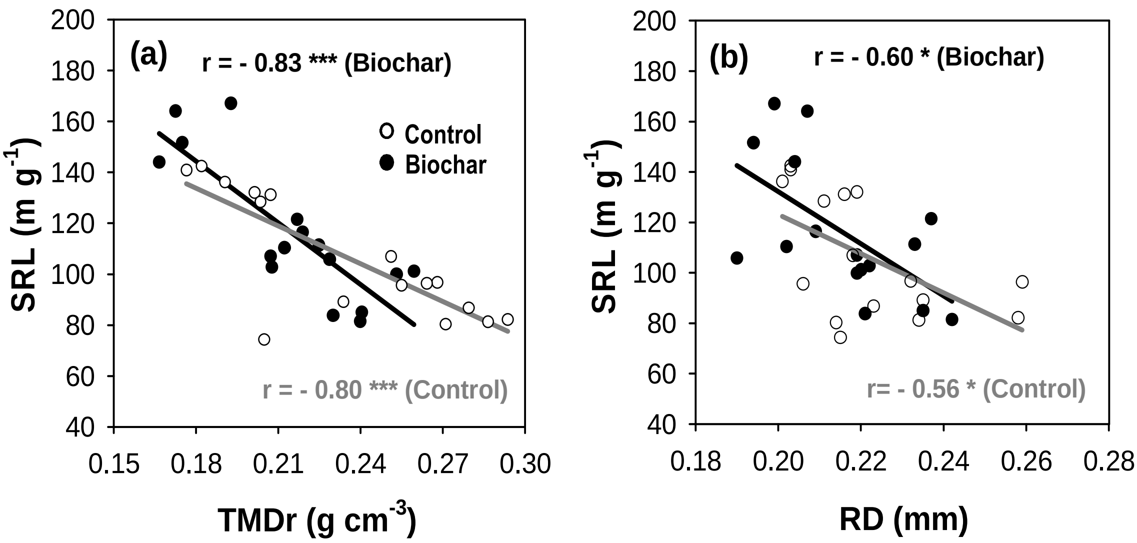

Fig. S1 Relationship between specific root length (SRL) and: **(a)** root tissue mass density (TMDr); **(b)** root diameter (RD) for control and biochar treatments. Pearson correlation coefficients and significance levels are given: * *P <0.05;* and *** *P <0.001*

Capítulo 5. Spatial heterogeneity of soil biochar content affects soil quality and wheat growth

Olmo M, Lozano AM, Barrón V, Villar R

REGULAR ARTICLE

Spatial heterogeneity of soil biochar content affects soil quality and wheat growth and yield

Manuel Olmo · Ana María Lozano · Vidal Barrón · Rafael Villar

Abstract

Biochar (BC) is a carbonaceous material obtained by pyrolysis of organic waste materials that has been proposed as a soil management strategy to mitigate global warming and to improve crop productivity. Once BC is applied to the soil, the imperfect and incomplete mixing of BC with soil during the first years and the standard agronomic practices (i.e. tillage, sowing) may generate a spatial heterogeneity of BC content in the soil which may have implications for soil properties and their effects on plant growth in small-scale studies.

We investigated how the spatial heterogeneity of olive-tree pruning BC applied to a vertisol after two agronomic seasons affected soil characteristics and wheat growth and yield. During the second agronomic season and just before wheat germination we determined the BC content in soil by an in-situ visual categorization based in the soil darkening, which was strongly correlated to BC content of soil and the soil brightness. We found a high spatial heterogeneity in the BC plots that affected the soil characteristics and wheat growth and yield. Patches with high BC content showed a reduced soil compaction and an increased soil moisture, pH, electrical conductivity and nutrient availability (P, Ca, K, Mn, Fe and Zn) and, consequently wheat had a higher relative growth rate, tillering and grain yield. However, if the spatial heterogeneity of BC content in soil was not taken into account, most of the effects of BC on wheat growth would not have been detected. Our study reveals the importance of taking into account the spatial heterogeneity of BC content.

Key-words field, leaf traits, relative growth rate, soil brightness, visual categorization, specific leaf area

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Introduction

Biochar (BC) is a carbon-rich material obtained by the thermal decomposition of organic matter under a limited supply of oxygen (pyrolysis) and at relatively low temperatures (Lehmann and Joseph, 2012). Due to its highly aromatic structure, BC is chemical and biologically more stable compared with the organic matter from which it was made. That means that the C contained in BC is not likely to degrade to CO_2 to the same rate as untreated organic matter. Therefore BC is used as a strategy to increase long-term soil C stocks and thus to sequester C (Lehmann, 2007; Sohi et al., 2010). The mitigation potential of BC has been estimated to be as high as 12% of current anthropogenic CO_2 emissions (Woolf et al., 2010).

BC is also used as a soil amendment to improve soil quality. BC benefits to soil have been attributed to the reduction of soil bulk density and leaching of nutrients, increase in soil cation exchange capacity, water-holding capacity and nutrient availability (Keith et al., 2011; Quilliam et al., 2012; Liu et al., 2013; Olmo et al., 2014; 2015). These changes may be responsible to the increases in plant growth and yield that has been found in different studies (Atkinson et al., 2010; Major et al., 2010; Olmo et al., 2014). Also, the several meta-analyses done up to the date (Jeffery et al., 2011; Biederman and Harpole, 2013; Liu et al., 2013), including many different studies, have found a general positive effect on soil quality and plant productivity, but, there is also a wide variability as the effect of BC on the productivity has been found to be null or negative. This variability seems to be related to many factors as soil type, BC characteristics, grown conditions, etc. (Jeffery et al., 2011; Biederman and Harpole, 2013; Liu et al., 2013).

Most of the studies that evaluates the effects of BC on plant productivity are done under controlled conditions (growth chamber or greenhouse) (Rajkovich et al., 2012; Zhang, 2012; Alburquerque et al., 2013, 2014) and less studies are done under field conditions but it is increasing in the last years. Although the experiments under controlled conditions have many advantages, the conclusions of these experiments cannot be translated to what will happen in field conditions. In that sense, Liu et al. (2013) found in its meta-analysis that the effect of BC application on plant productivity under field conditions is lower to that found under controlled conditions. Therefore, it is necessary to do experiments under field conditions to understand the effects of BC application in real conditions on plant productivity and many aspects of ecosystem functioning (Biederman and Harpole, 2013; Liu et al., 2013).

Under field conditions, once BC is applied to soil, the mixing of BC with soil during the subsequent years and the agronomic practices for soil management as tillage and sowing may determine a heterogeneous distribution of BC in the soil. This may be an important factor because most of these studies are based on experimental design that includes small plots (from 18 to 25 m^2). Most of the field studies that evaluate the short term effects of BC do not consider spatial changes in BC content in soil along the time due to these agronomic practices. As a result of the heterogeneous distribution of BC in the soil, crop responses may not be so clear. In that sense, Liu et al. (2013) found that the BC effects on plant productivity are clear and significant during the 1st and 2nd year after BC application, but the effects became non-significant during the 3rd and 4th year after application of BC.

Therefore, analyses of BC content in the soil along the time and space, as well as a fast and accurate method for quantification of BC content in soil become necessary. The difficulty to distinguish BC from other forms of organic matter has resulted in methods that are either extremely labor intensive or require specialized instrumentation (Manning and Lopez-Capel, 2009; Nguyen et al., 2009). We propose a fast method to estimate BC content in soil based in an in-situ visual categorization of patches, due to the darkening that BC confer to soil. These visual categories can be contrasted with the measurement of BC content of soil, based in the loss-on-ignition method and the measurement of the soil brightness by diffuse reflectance spectroscopy.

To understand why BC addition increases plant production, it would be interesting to unravel the causes of the increase of plant growth by studying the changes in functional traits related to growth. Functional plant traits are attributes that influence the plant vital rates (e.g. growth and yield) and can be a useful proxy to predict plant functioning (Lavorel and Garnier, 2002; Cornelissen et al., 2003). Most of the field studies on BC are focused on yield and did not study the BC effects on important functional plants traits that affect growth and could be the cause of the higher yields. This is another novelty of our study, as we focus in some leaf functional traits related with growth as the size and specific leaf area of the flag-leaf. Several studies report that functional traits of the flag-leaf are reliable indicators of inherent relative growth rate and grain yield in wheat, as the flag-leaf size and thickness were positively related with grain yield (Araus et al., 1986, 1987; Shearman et al., 2005).

Our field study is also in an area (the Mediterranean region) where there is a lack of field studies (Liu et al., 2013). Considering that Mediterranean areas are limited by water and nutrients and that the effect of BC addition seems to be to increase the field water capacity and the availability of nutrients (Baronti et al., 2014; Tammeorg et al., 2014), it would be interesting to explore the effects of BC application in this area.

Therefore, the aims of this study were: 1) to determinate if there is spatial heterogeneity in BC content in the soil after one season of its application; 2) to test appropriate methodologies to estimate BC content in soil; and 3) to evaluate the effect of BC content on soil properties and wheat growth and yield. We hypothesized that: (i) BC content distribution is heterogeneous after one season of its application; (ii) several methodologies (fast and not expensive) could be applied to determine the BC content in soil; and (iii) BC content affects to soil properties and to key functional plant traits and, as a result, plants in BC-rich soil patches show a higher wheat growth and grain yield. The main novelty of our study compared with previous field studies lies in the study of spatial heterogeneity of BC content in soil in plots where BC was added and its effects on soil properties, key leaf functional traits and plant growth and yield.

Materials and Methods

Experimental design

The field experiment was conducted over two consecutive growing seasons (2012/2013 and 2013/2014) at the "Origuero" farm located near Córdoba (southern Spain) (37° 49' 47" N, 4° 44' 32" W, 215 m above sea level). The soil, classified as a Vertic Calcixerert (USDA Soil Taxonomy) had a clay-loam texture (24% sand, 38% silt, and 38% clay), pH 7.9 (1:2.5 soil:water), electrical conductivity of 0.1 dS m^{-1} (1:5 soil: water), 6.9 g kg^{-1} C_{org}, 320 g kg^{-1} calcium carbonate equivalent and 11 mg kg^{-1} Olsen-P.

BC was applied unmilled to the soil at the rate of 4 kg m^{-2} (40 Mg ha^{-1}, aprox. 2 % w/w on a dry mass basis) on April 24, 2013 and incorporated into the top 20 cm soil depth immediately, doing the best to have an uniform distribution. The BC had an alkaline pH value of 9.4, electrical conductivity (EC) of 1.4 dS m^{-1} (1:10 BC/water), cation exchange capacity of 30 $cmol_+ kg^{-1}$ and 0.8, 5.4, 86.0, and 2.0 g kg^{-1} of P, K, Ca, and Mg, respectively. Additional details about the properties of BC and the analytical methods for its characterization can be found in Alburquerque et al., (2013, 2014).

The experimental design consisted of a randomized complete block with plots of 16.8 m^2 (6 × 2.8 m) considering two treatments: control (without BC addition), and soil treated with BC. There were four plots per treatment, resulting in a total area of 67.2 m^2 for each. Plots were separated from each other by 3 m (Fig. 1a).

After BC application, sunflower (*Helianthus annuus* L) was sowed and developed for four months (April 24, 2013 to August 27, 2013). At the beginning and at the end of crop season all plots were geo-referenced with a GPS (Garmin etrex, Garmin Ltd., Olathe) for later location of plots. Four months after sunflower harvest, soil was ploughed and wheat was sowed (December 18, 2013), using 20 cm row spacing, and grown for seven months (until June 9, 2014). The sunflower-wheat rotation system is the most common cropping system under non-irrigated conditions in Mediterranean climate at southern Spain. After planting wheat, a nitrogen fertilizer (urea) was top dressed at the rate of 290 kg ha^{-1} on December 18, 2013. Chemical herbicides [20 g ha^{-1} of a mixture of Ioxinil (7.5%) + Bromoxinil (7.5%) + MCPP (37.5%) and 0.8 L ha^{-1} + Accurate (Tribenuron 75%)] were applied on March 13, 2014.

During the wheat growing season, the average temperature was 14.7°C (mean of 7 months, December 2013 to June 2014), with a maximum temperature of 42 ºC in May, and an average relative humidity of 64% (data logger PCE-HT71, PCE-Instruments, Tobarra, Spain) (Fig. S1 in *Supporting information*). The total rainfall for the crop season was 340 mm (from December 18, 2013 to June 9, 2014) and the agronomic year was dry, with a total rainfall of 390 mm (from September 1, 2013 to August 31, 2014), which is 62 % of the average for the 1902-2013 period (Agencia Española de Meteorología, www. aemet.es/es/portada).

Spatial distribution of BC in the soil

After wheat was sown and before its germination a visual inspection of the BC plots gave the impression of a heterogeneous distribution of BC. Therefore, we did a visual categorization of the BC content in the soil. In each BC treated plots, 84 digital photographs were taken, each of these covering an area of 0.5 × 0.4 m. We used a digital camera (Nikon Coolpix S220, Nikon Corp., Japan) mounted on a tripod manufactured by ourselves (Fig. S2 in *Supporting information*). The photographs were taken during the morning of the same day, with the same orientation, height and similar light exposition. To avoid differences in light exposure that would induce errors in the assessment of BC particles (e.g. shadows), the lateral incident of light was avoided by covering with a sun shield. Later, the photographs were visualized on a PC and the categorical values were assigned based on the presence of BC particles in the photographed soil area. A value of 1 indicates a very low content, value 2 for a medium content, 3 for a high content and 4 for the highest content of BC (Fig. 1b). A value of 0 was assigned to plots non-treated initially with BC and that did not show any BC particles (Fig. 1b).

Determination of BC content in soil

To determine the exact BC content and its spatial distribution in the soil, we rely on two important characteristics that BC confers to soil after its addition; the increase of C content (determined by the loss ignition method) and darkness (determined by reflectance). For that, two soil samples (0-20 cm depth) were taken per visual-BC category (1, 2, 3 and 4) and plot. Two grams of these samples were calcined in a muffle at 450 ºC for 12 hours and the weight loss on ignition percentage (LOI_{sample}%) was calculated as: [(W_1-W_2)/ W_1], where W_1 was the sample weight before ignition and W_2 was the sample weight after ignition. In order to determinate the C content which corresponds only to the BC fraction, the $LOI_{control}$%, corresponding to the soil control (without BC) was subtracted and corrected taking into account the weight loss on ignition for the pure BC, estimated in a 75.6%, according to previous analysis. So, the following equation (Eq. 1) was applied:

$$BC\% = (LOI_{sample}\% - LOI_{control}\%) \times 100 / 75.6$$

A sub-sample of the above field-soil samples was used to determine their brightness by diffuse reflectance spectroscopy. The reflectance spectrum of the soil samples was acquired at intervals of 0.5 nm in the range 380-710 nm range with a UV-Vis-NIR Spectrophotometer Cary 5000 (Varian Inc., Palo Alto, California), equipped with a diffuse reflectance attachment. Then, the spectrums were converted to tristimulus values (X,Y,Z), according to the CIE colour-matching spectral energy distribution weighted for CIE Source C (Wyszecki and Stiles, 1982) and finally transformed to the Munsell hue value (or brightness) and chroma, using the method given by the Munsell conversion program (www.munsell.com). Brightness is a non-dimensional variable.

Determination of soil variables

Soil penetration resistance was measured monthly at the 0-20 cm depth in each BC content category and plot (a total of 8 repetitions for each visual-BC category), using a penetrologger probe (Eijkelkamp, Giesbeek, The Netherlands), with a cylindrical probe of 3.3 cm^2 and 30° cone angle. In the same site two soil samples were taken for gravimetric water content. Mean values for soil compaction and moisture for each visual-BC category and the whole experiment were calculated. For this, data of both variables were graphically represented and the areas under the curves were calculated using Adobe Photoshop CS5 (Adobe Systems, Inc., EEUU). The obtained area ($pixels^2$) was divided by x-axis longitude (pixels) and multiplied by the ratio variable dimensions/pixel (in y-axis), obtaining the mean value of soil compaction (MPa) and gravimetric water content ($g\ g^{-1}$).

For chemical analyses, at the end of the experiment, three replicates of soil samples (0 - 20 cm depth) per each visual-BC category and plot were taken randomly and mixed to form a composite sample for each category and plot. Each sample was sieved to <2 mm for pH, EC, and available nutrient concentration. The pH was determined in a 1:2.5 (w/v) soil/water extract using a pH meter and EC in a 1:5 (w/v) soil/water extract using a conductivity bridge. The available soil P was extracted with 0.5 M $NaHCO_3$ (1:10, w/v) for 30 min and measured colorimetrically ("Olsen P"; Olsen and Sommers, 1982). Available Mg and K were extracted with 1 N NH_4OAc and available Fe, Mn, Cu and Zn with 0.05 M DTPA and all were determined by atomic absorption spectrophotometry. In addition, for control soil, the $CaCO_3$ content was determined by the Wesemael method and the soil organic C was determined according to the Walkley-Black method (Walkley and Black, 1934).

Plant sampling

Seventy days after wheat sowing (DAS, February 26, 2014), two areas of 25 × 25 cm per each visual-BC category and plot were randomly selected to sample wheat plants. Plants were harvested at ground level and in the laboratory, the number of tillers was counted and they were oven-dry (at 70°C for 72 h), then the dry mass was weighed.

The second sampling was conducted on May 9, 2014 (142 DAS). Crop was in post-anthesis stage and grain was in early dough stage (Zadoks et al., 1974). The methodology was similar to that of the first sampling by randomly sampling two areas (but larger, 40 × 50 cm) per visual-BC category and plot. Plants were harvested at ground level and in the laboratory, the number of tillers was counted and were separated into spike, flag-leaf, leaves, and stems and oven-dry (at 70°C for 72 h), then the dry mass of these fractions was determined. In addition, the flag-leaves of 8 individual plants were randomly selected and scanned. The area of the flag leaves was measured with the software Image Pro v4.5 (Media Cybernetics, Atlanta, GA). Finally, the leaves were oven-dried at 70º C for 72 h and weighed.

The third sampling was performed on June 9, 2014 (173 DAS). Crop was in the ripening stage (Zadoks et al., 1974). To evaluate the effect of BC on final wheat biomass and grain yield we followed the same methodology as in the previous samplings, but in this case three areas of 40 × 50 cm per visual-BC category and plot were randomly selected. In the laboratory, the aerial part was separated into spikes and grain to obtain the dry mass (at 70°C for 72 h).

With these data we calculated several plant variables. The specific leaf area of the flag-leaf ($SLA_{Flag\text{-}leaf}$) was calculated as the leaf area divided by the leaf dry mass. Relative growth rate of the aboveground fraction (RGR_{shoot}) was calculated as: $(\ln M_2 - \ln M_1) / (t_2 - t_1)$ (Villar et al., 2008), where M_1 and M_2 were the aboveground dry mass at times t_1 and t_2 (first and second sampling, respectively).

Statistical analysis

Data were analysed with a one-way ANOVA for a randomized complete block design, with visual-BC category as factor. To analyse differences between BC content categories, a Tukey post-hoc test was used with a *P* level of 0.05. Data were transformed if the variables did not fulfill the assumptions of ANOVA [in second sampling RGR_{shoot} and spike biomass were log transformed]. Spearman correlations were used to determine the relationships between variables. The programs STATISTICA™ v.8 (Statsoft, Inc., Tulsa, USA) and Statistix v.9 (Analytical Software, Tallahassee, FL, USA) were used for statistical analysis.

Results

Methods to determinate the spatial heterogeneity of BC content in soil

The in-situ visual categorization of the digital photographs of the soil revealed that BC content in soil was heterogeneous and its spatial distribution was not uniform (see Fig. 1c for an example). In plots treated initially (during 2012/13 season) with BC we found a season after (2013/14) that there were soil patches with a high BC content (category 4 and 3), and others with a medium and low BC content (category 2 and 1, respectively).

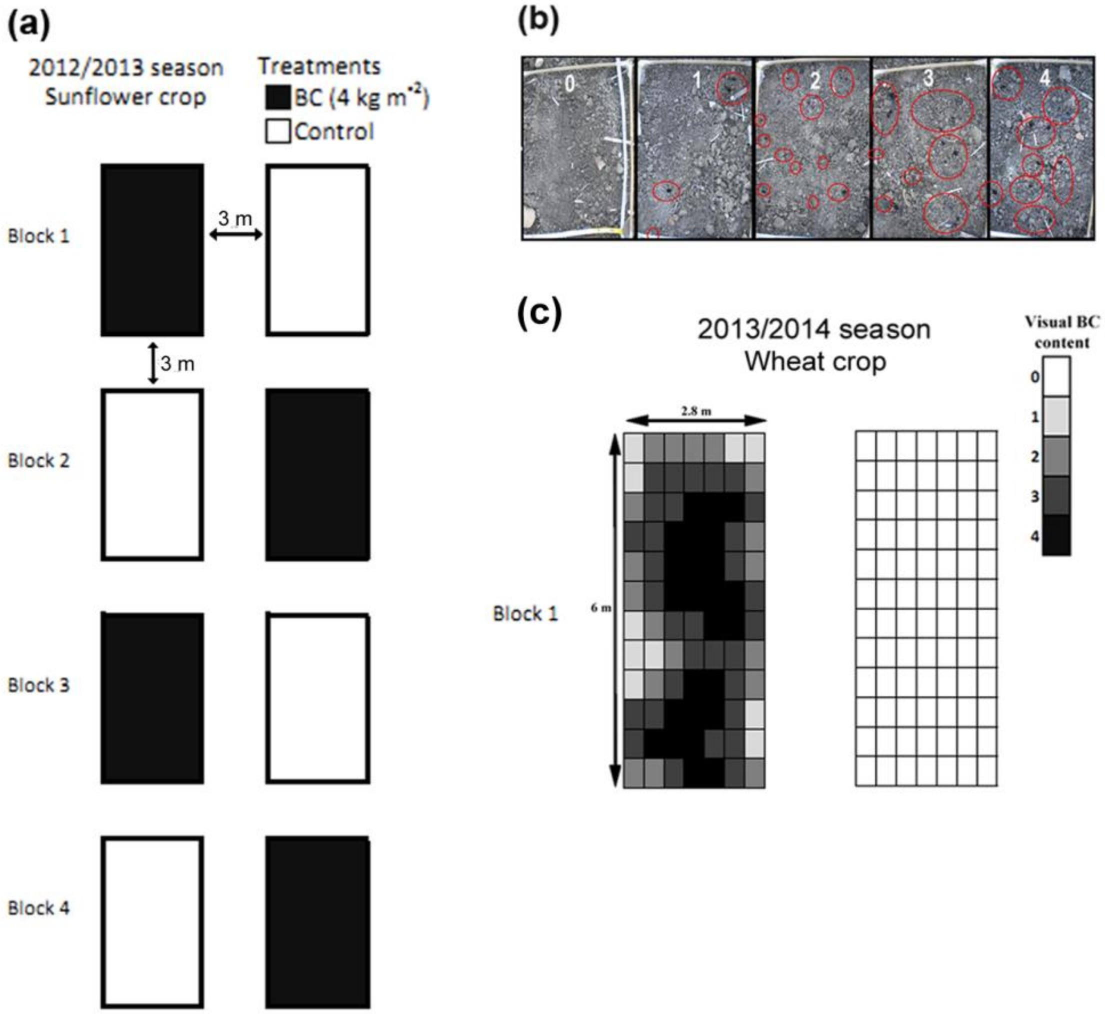

Fig. 1 (a) Illustration of the experimental design during the previous season (2012/2013). **(b)** Categorization of photographs based on the presence of biochar (BC) particles (2013/2014 season). A value of 1 indicates a very low content of BC and a value of 4 the highest content of BC. We assigned a value of 0 to plots non-treated with BC initially (2012/2013 season) and that did not showed any BC particles. **(c)** Illustration of the visual-BC categories in soil in block 1. To the left the current spatial distribution of BC in a plot initially treated with BC and to the right the control plot (without BC amended). Each cell (0.5 × 0.4 m) corresponds to a digital photograph taken on field for the visual categorization of BC content. For interpretation of BC content see the colors legend

As it was expected, plots non-treated initially with BC (control treatment in the previous season) did not show any BC particles (category 0) a season after.

The visual-BC category was positively and strongly related with the BC content determined by the loss on ignition method (Fig. 2a). Furthermore, the visual-BC category was negative and strongly related with soil brightness (Fig. 2b). BC content measured with the loss on ignition method was negative related with soil brightness (r_s = -0.84, $P < 0.001$; data not shown).

Effect of the spatial heterogeneity of BC on soil variables and crop development

The high heterogeneity of the BC distribution in plots with BC added a season before had a contrasting effect on several soil properties such as soil compaction, moisture and nutrient availability. Patches with high BC content showed a lower soil compaction and high soil moisture (Fig. 3a, b, Table S1, S2 in *Supporting information*).

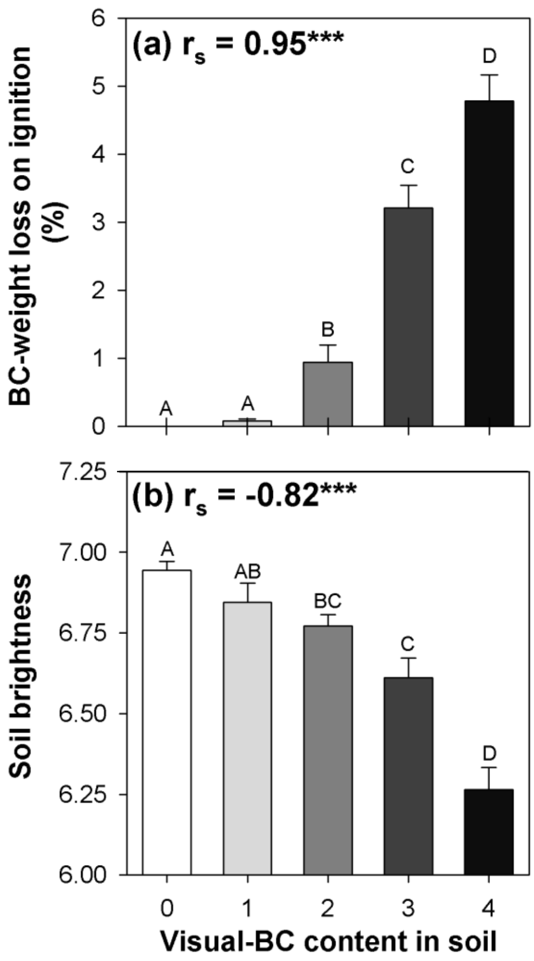

Fig. 2 Mean ± SE (n= 8) of **(a)** percentage of the biochar content in the soil samples measured by the loss on ignition method; and **(b)** soil brightness, for each visual-biochar (BC) category. Different letters indicate significant differences between visual-BC categories (Tukey's hsd post hoc tests, *P* <0.05). Spearman correlation coefficient and significance is given (*** *P* <0.001)

Similarly, patches with high BC content had a high pH, EC, macronutrients (Ca, K, Mg and available P) and micronutrients (available Mn, Fe and Zn) (Fig. 3, Table S3 in Supporting information). All these soil characteristics showed significant relationships with visual BC-content categories.

The heterogeneity of the BC distribution in plots with BC added a season before had also a contrasting effect on several variables related to crop development. At the second sampling (142 DAS), an increase in BC content was significantly related to an increase in plant tiller density, aboveground plant biomass and spike biomass (Fig. 4a, b, c), and leaf and stem biomass (data not shown). The plants in patches with high BC content showed higher RGR_{shoot} (Fig. 4d). Respect to the flag-leaf, plants in high BC content patches had an increase in the size and a decrease in its specific leaf area ($SLA_{Flag\text{-}leaf}$) (Fig. 4e, f). At the third and final sampling (173 DAS), spike biomass were significantly increased with the visual-BC content (r_s = 0.47, *P* < 0.001; data not shown). Also grain production and spike biomass fraction were significantly increased with visual BC content (Fig. 4g, h).

As a summary we showed a network of significant relationships between the visual BC content and its effects on soil properties that may affect plant development (Fig. 5). The BC content of soil was correlated positively with soil moisture, pH and nutrient availability (the total content of macro- and micro-nutrients), and negatively with soil compaction. Nutrient availability and moisture were also positively correlated, and moisture and soil compaction were negatively correlated. Soil moisture and nutrient availability were positively correlated with aboveground plant biomass (at 142 DAS) and grain production, whereas soil compaction was negatively correlated with them. A higher aboveground plant biomass was related to a higher grain production. Furthermore, leaf traits of the flag leaf were associated with aboveground biomass and grain production, positive relationships with flag-leaf size and negative relationships with the specific leaf area of the flag-leaf ($SLA_{Flag\text{-}leaf}$) (Fig. 5).

Discussion

Our results demonstrate that the high spatial heterogeneity in the distribution of BC content in the soil induces a different effect on soil properties that affect in a different way the growth and grain yield of wheat. Therefore, the effect of management practices (related to tillage, sowing, etc.) should be taken into account when the effect of BC in field studies along several years want to be studied.

Methods to quantify the spatial heterogeneity of BC content in soil

We present several methods to quantify the spatial distribution of BC content in soil. First, we present a fast and easy method to apply in a field situation: a visual categorization of BC content in soil. The only instrument we need is a digital camera mounted in a custom tripod (made with bamboo sticks), therefore a very cheap equipment. It is also very quick, as in one morning two persons can do about 336 photographs (the four plots of 6 × 2.8 m).

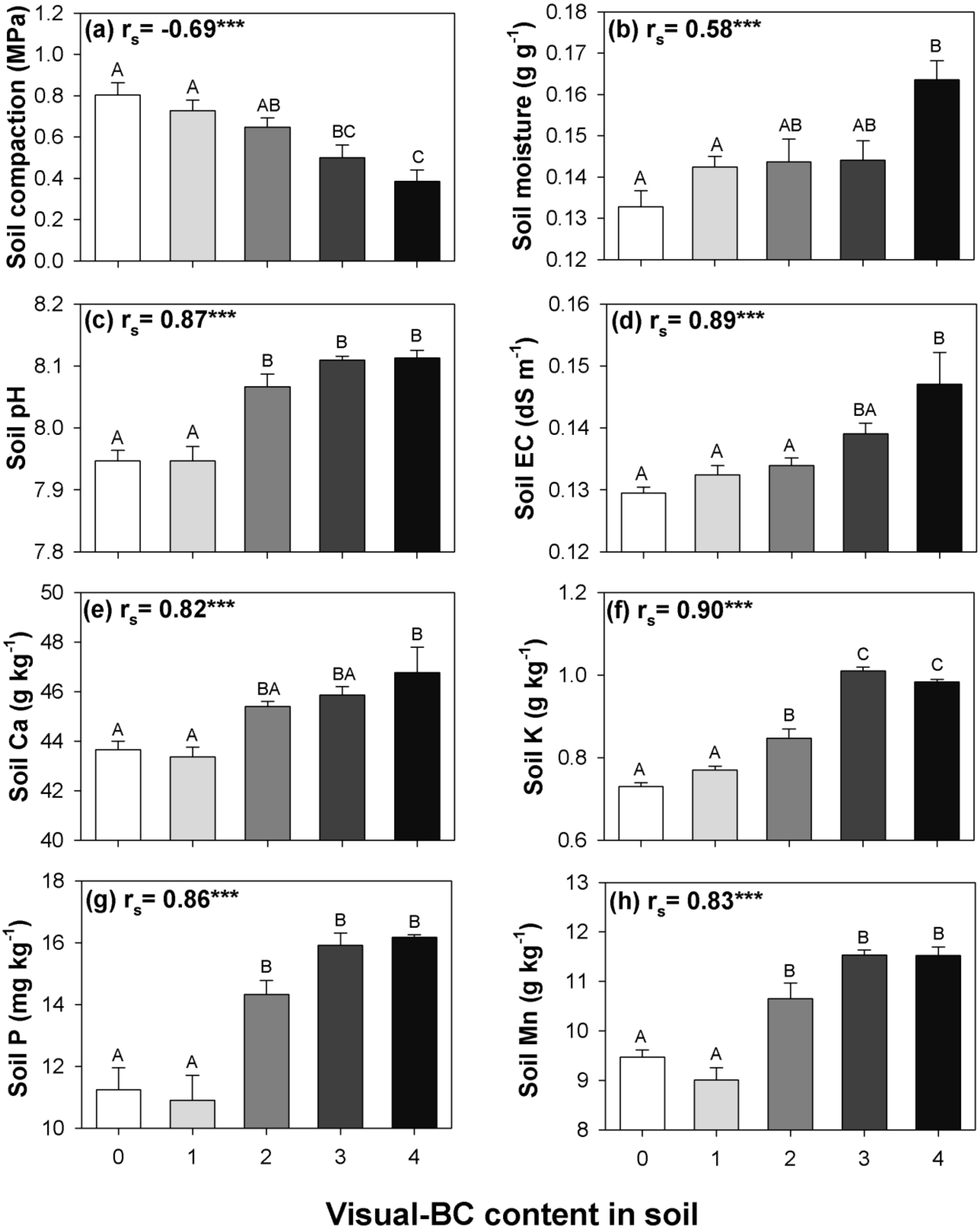

Fig. 3 Effects of the spatial heterogeneity of biochar (BC) content in soil on some soil variables (mean ± SE). Soil compaction and moisture (both measured on March, 27; 99 days after sowing, n= 8), pH (n= 4), electrical conductivity (EC, n= 4) and soil nutrients availability (Ca, K, P and Mn, n= 4). Different letters indicate significant differences between visual-BC categories (Tukey's hsd post hoc tests, P <0.05). Spearman correlation coefficient and significance between each variable and the visual-BC category is given (*** P <0.001)

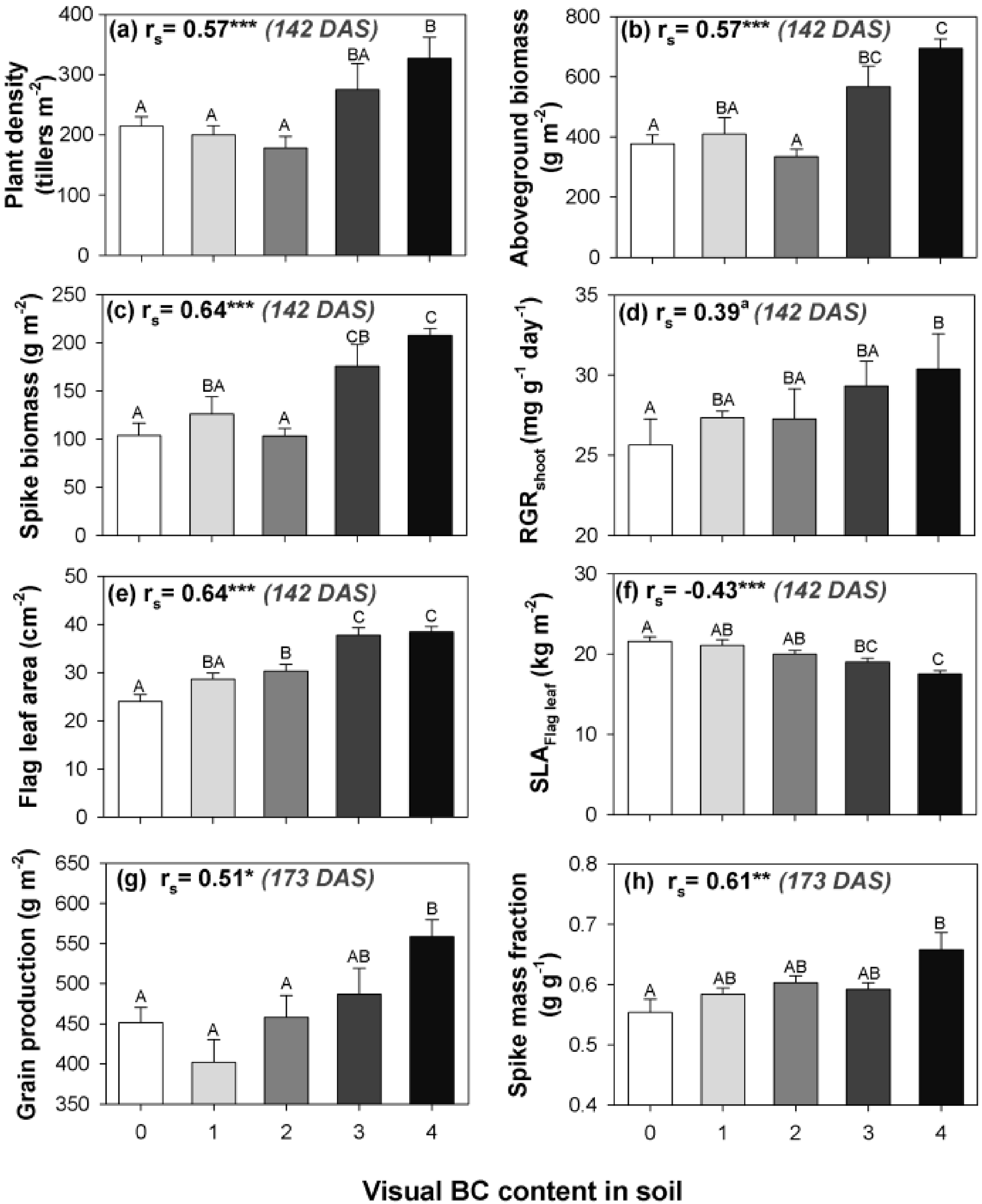

Fig. 4 Mean ± SE (n= 8) of **(a)** plant density, **(b)** aboveground biomass, **(c)** spike biomass, **(d)** Relative growth rate of aboveground portion of the crop (RGR_{shoot}), **(e)** flag-leaf area and and **(f)** specific area of the flag-leaf ($SLA_{Flag\text{-}leaf}$)**,** at 142 days after sowing (DAS); and **(g)** grain production and **(h)** spike biomass fraction (proportion of spike respect to aboveground biomass), at 173 days after sowing (n=12), for each visual-biochar (BC) content. Different letters indicate significant differences between visual-BC categories (Tukey's hsd post hoc tests, *P* <0.05). Spearman correlation coefficient and significance between each variable and the visual-BC category is given: ([a] 0.1> *P* >0.05; * *P* <0.05; ** *P* <0.01 and *** *P* <0.001). The flag-leaf area and $SLA_{Flag\text{-}leaf}$ were evaluated at the individual plant level (n= 40)

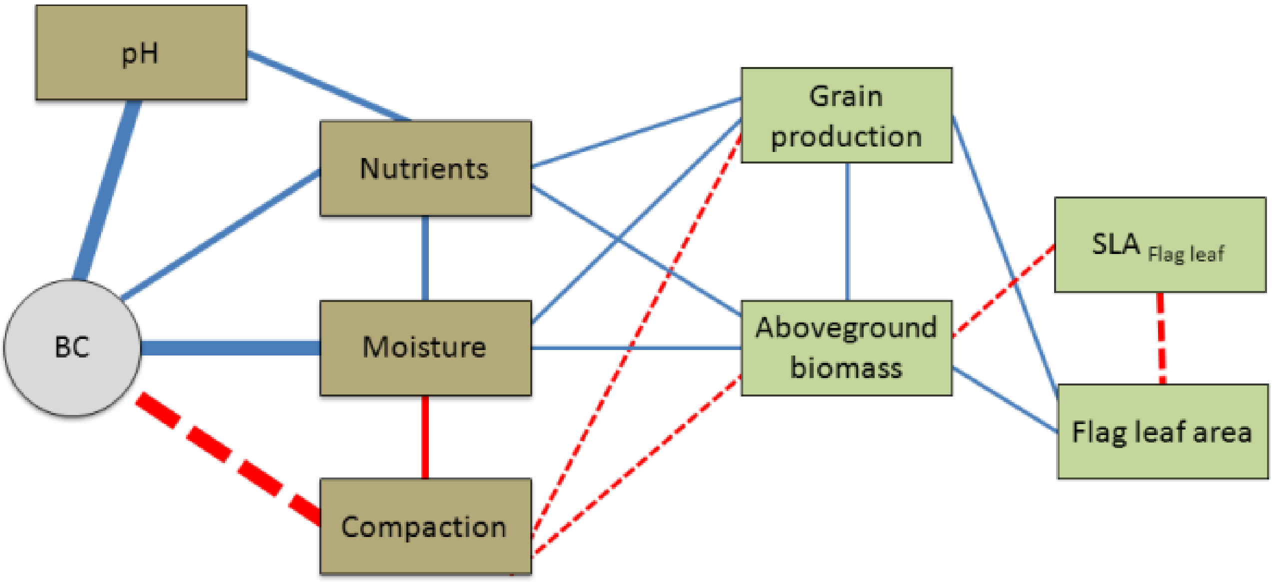

Fig. 5 The correlation network of soil (brown boxes) and plant variables (green boxes). Blue continuous lines indicate positive correlations and red dashed lines negative correlations. Thin lines explain between 25–50% of the variation (0.50< |r| <0.71), intermediate lines 50–75% (0.71< |r| <0.87) and thick lines>75% of the variation (|r| >0.87). In order to simplify the current correlation network we used the mean of soil moisture and compaction, for each visual BC category (calculating the areas under the curves for the whole experiment). For nutrients we calculated the total content of macro- and micro-nutrients (P, Ca, K, Mg, Fe, Mn, Cu and Zn)

It is advisable that the categorization of BC content after the view of photographs in the computer was made by two persons, as it would be more objective. This apparently subjective method was strongly correlated to the BC content measured with the loss ignition method and with soil brightness. Therefore, we advise the visual categorization for a fast and easy method to determine the heterogeneity of BC in soil, and if a more exact determination of BC content is required the loss ignition method can be performed in some samples covering a wide range of the visual BC content categories.

Effect of the spatial heterogeneity of BC content in soil characteristics and crop development

The high spatial heterogeneity of BC in soil had caused substantial and significant differences in soil physical and chemical characteristics. BC content significantly reduced soil penetration resistance during the whole experiment. Similar results have been found in other studies (Olmo et al., 2014; Rogovska et al., 2014; Tammeorg et al., 2014). The high internal porosity and low bulk density of BC (Brewer et al., 2009) generally implies increased pore volume that can decrease soil compaction. This reduction may be beneficial for plant growth, since high levels of compaction adversely affect root growth reducing the soil volume exploited (Alameda and Villar, 2012; Nosalewicz and Lipiec, 2014) and therefore reducing the supply of nutrients and water to the plant (Masle and Passioura, 1987; Atwell, 1990; Oussible et al., 1992).

On other hand, soil moisture was significantly increased with the BC content during the whole crop season. Most of the studies have found this result (Karhu et al., 2011; Baronti et al., 2014), but also it has been found recently (Jeffery et al. 2015) that the capacity to increase the soil water retention with BC seems to be dependent of the type of BC and the hydrophobic nature of it. In most of the cases, the increasing water field capacity with BC addition can be associated to the high porosity of BC, and this have been described as one of the principal benefits of its use as a soil amendment (Makoto and Yasuyuki, 2010; Verheijen et al., 2010; Abel et al., 2013; Olmo et al., 2014). Although our results refer to the total amount of water in the soil, recent studies have confirmed that the water retained in BC pores is available for plants (Brockhoff et al., 2010; Baronti et al., 2014). In our study, this seems to be an important factor as soil water is often the most limiting factor for crop production in the Mediterranean region (Bennet et al., 1998). In addition, as the current year was considered as a dry year (a reduction of about 40% of rainfall), an increase in soil water content by BC addition could play a decisive role for crop development in our study.

We also found that soil nutrient availability was increased with the increase of BC content, as it has been found in other studies (Biederman and Harpole, 2013; Olmo et al. 2015). The increased of P, Ca and K content of the soil could be attributable to the high proportion of these nutrients in BC (Major et al., 2010; Tammeorg et al., 2014). The increase in soil Olsen-P with the increase of BC content is a relevant result since calcareous soils, as in this study, are usually deficient in available P due to the precipitation of Ca-rich phosphates and sorption processes (Solis and Torrent, 1989). Other studies (Alburquerque et al., 2013; Biederman and Harpole, 2013; Olmo et al., 2014) have also found that BC addition to soil increase P availability. All together, the reduction of compaction, the increase of soil moisture and the nutrient availability seems to have beneficial effects for crop development. These improvements may create a more favorable root growth environment, and therefore increasing the ability of the plants to utilize available nutrients and water (Olmo et al., 2015).

Interestingly, a higher BC content increases the tillering of wheat. The grain yield depends of the number of tillers surviving up to maturity. Production and survival of tillers is dependent of the environmental conditions, in particular of water and nutrients availability (Simane et al., 1993; Liakas et al., 2001), suggesting that the increase of tillering was due to the soil improvements after BC addition.

Our results showed that plants growing in soils with BC had changed its functional traits related to the flag leaf (an increase in size and a decrease in SLA). Several studies (Austin et al., 1977; Sylvester-Bradley et al., 1990) have found that the flag-leaf makes a major contribution to grain yield in wheat, which can be explained by a greater photosynthetically active surface area. In this sense, Shearman et al. (2005) showed that the yield improvement in the United Kingdom was associated with a reduced SLA which is related to a higher flag-leaf size. Rawson et al. (1987) found that thicker flag-leaves and with low SLA may be indicative of higher wheat growth.

Similar results were reported by Olmo et al. (2014), who found that BC-treated plants showed a reduction of $SLA_{Flag\text{-}leaf}$ which was related with a higher plant growth rate. A SLA reduction has been related with an increase in the leaf photosynthetic efficiency due to a likely increase in the amount of photosynthetic machinery per unit leaf area (Reich et al., 1998). Also, increases in photosynthetic activity after BC addition have recently been related to the alleviation of water and disease stresses (Baronti et al., 2014; Wang et al., 2014).

The positive effects of BC addition on crop development were also obtained at the final harvest, with a higher aboveground biomass, spike biomass and grain production with increasing BC content in the soil. The increase in the crop yield can be due to the improvement of soil properties, caused by BC during grain filling phase, as the greater water and nutrients availability. The influence of BC on wheat yield in the field is complex and greatly depends on the interactions of soil improvements (physicals and chemicals) by BC addition. It is difficult to know which factor is more important in determining the increase in wheat growth and yield, as all these factors varies in a correlated way with the BC content in the soil (Fig. 5). Therefore, it would be necessary to perform different experiments to isolate the different factors that change with BC addition and are the main cause of the increase in plant growth. Our results suggest that from all soil changes due to BC addition, the increase in water and nutrients availability seems to be the factors that determine in a greater extent the increase of crop development and yield (Fig. 5).

The different soil BC content found in our study can also be considered as an experiment of different doses of BC in field conditions. Our results show that an increase in BC content (from 0 to 5% w/w, at level 4 of visual BC content) has a positive effect on various soil properties (moisture, compaction, nutrient availability, etc.) that increase growth and wheat yield. The review of Biderman and Harpole (2013) found a non-clear effect of BC application rate and aboveground productivity, with some studies showing a positive effect but other with null or negative effect of increasing BC application rate.

Other studies have found that the addition of BC did not have a positive effect on wheat grain production (Karer et al., 2013). However, the review of Liu et al. (2013) found that the addition of BC in wheat crops could increase production by about 10%, slightly lower than that found in our study (about 22%). These different results may be due to differences in soil type, growing conditions and type of BC (Jeffery et al., 2011; Liu et al., 2013). But also, the cause of not finding an effect of BC on plant productivity could be due to other factors including the spatial heterogeneity of BC in the soil. In that sense, considering our experiment, when analyzing plant variables without regard the spatial heterogeneity of BC content in soil, i.e. considering only two treatments: BC treatment (for plots initially treated with BC) and control treatment (for plots non-treated with BC initially), results revealed that BC addition affects significantly only three of the eleven evaluated plants variables. Only spike biomass (at 142 DAS), spike biomass fraction (173 DAS) and flag-leaf area differed significantly between both treatments (Table S4 in *Supporting information*). Hence the importance of considering the spatial heterogeneity of BC content in soil, since if it is not taken this into consideration, the crop responses may not be clear.

Conclusions

In field studies based on experimental small plots, once biochar is applied to soil, its imperfect mixing with soil during the first years and the traditional agricultural practices, may generate a spatial heterogeneity of its content in soil. The proposed in-situ visual categorization revealed that biochar content in soil was heterogeneous and had changed since its application to soil. Wheat crop responded to the heterogeneity of biochar content, plants increased the growth and grain yield in biochar-rich soil patches. Most of the medium-term field studies that are based on experimental small plots seem to do not consider these changes in the spatial distribution of biochar content, which may result that crop responses to BC addition could not be very clear. Therefore, field studies should consider the spatial heterogeneity of BC content in soil.

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Supporting information

Table S1 Soil compaction (MPa; mean ± SE, n= 8) of the visual-biochar categories in soil (0, 1, 2, 3 and 4) at 23, 76, 99 and 143 days after sowing (DAS). For each date, we showed the Spearman correlation coefficient between compaction for each date and the visual-biochar categories and significance (** *P* <0.01; and *** *P* <0.001). Significant values are written in bold font

Date	**Visual-biochar categories**					r_s
	0	**1**	**2**	**3**	**4**	
January, 10 (23 DAS)	0.91 ± 0.07	0.75 ± 0.05	0.80 ± 0.05	0.81 ± 0.06	0.66 ± 0.05	**-0.41*** **
March, 4 (76 DAS)	0.55 ± 0.02	0.34 ± 0.01	0.33 ± 0.02	0.24 ± 0.03	0.26 ± 0.01	**-0.82*****
March, 27 (99 DAS)	0.80 ± 0.06	0.73 ± 0.05	0.65 ± 0.05	0.50 ± 0.06	0.38 ± 0.06	**-0.69*****
May, 9(143 DAS)	0.84 ± 0.14	0.69 ± 0.06	0.70 ± 0.04	0.51 ± 0.05	0.35 ± 0.03	**-0.79*****

Table S2 Soil moisture (g g^{-1}; mean ± SE, n= 8) of the visual-biochar categories in soil (0, 1, 2, 3 and 4) at 23, 76, 99, 143 and 174 days after sowing (DAS). For each date, we showed the Spearman correlation coefficient between soil moisture and the visual-biochar categories and the significance (*** *P* <0.001). Significant values are written in bold font

Date	**Visual-biochar categories**					r_s
	0	**1**	**2**	**3**	**4**	
January, 10 (23 DAS)	0.26 ± 0.01	0.27 ± 0.01	0.28 ± 0.01	0.28 ± 0.01	0.30 ± 0.01	**0.61*****
March, 4 (76 DAS)	0.30 ± 0.01	0.31 ± 0.01	0.30 ± 0.01	0.32 ± 0.01	0.33 ± 0.01	**0.54*****
March, 27 (99 DAS)	0.13 ± 0.01	0.14 ± 0.01	0.14 ± 0.01	0.14 ± 0.01	0.16 ± 0.01	**0.58*****
May, 9 (143 DAS)	0.06 ± 0.01	0.08 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	**0.83*****
June, 9 (174 DAS)	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	**0.87*****

Table S3 Available nutrients (mean ± SE, n= 4) of the visual BC categories in soil (0, 1, 2, 3 and 4), at the end of experiment (174 DAS), values for nutrient concentration are given on a dry weight basis. For each date, Spearman correlation coefficient between each variable and the visual-biochar categories and significance is given (ns *P* >0.05; and *** *P* <0.001). Significant values are written in bold font

Variable	**Visual-biochar categories**					r_s
	0	**1**	**2**	**3**	**4**	
Mg (g kg^{-1})	0.63 ± 0.02	0.60 ± 0.01	0.60 ± 0.01	0.63 ± 0.03	0.69 ± 0.01	0.39 ns
Fe (mg kg^{-1})	2.04 ± 0.04	2.13 ± 0.03	2.12 ± 0.01	2.26 ± 0.01	2.36 ± 0.03	**0.91*****
Cu (mg kg^{-1})	1.61 ± 0.03	1.57 ± 0.02	1.43 ± 0.15	1.58 ± 0.07	1.73 ± 0.04	0.42 ns
Zn (mg kg^{-1})	0.45 ± 0.02	0.45 ± 0.02	0.52 ± 0.02	0.51 ± 0.01	0.58 ± 0.03	**0.83*****

Table S4 Plant variables (mean value ± SE) measured for control (plots non-treated with biochar initially) and biochar treatments (plots initially treated with biochar), without regard the spatial heterogeneity of biochar content in soil. The levels of significance (ns P >0.05, * P <0.05 and ** P <0.01) are indicated. Significant values are written in bold font

Variable	Control	Biochar	*P*
Plant density (tillers m^{-2})	214 ± 16	245 ± 18	ns
Aboveground biomass (142 DAS) (g m^{-2})	377 ± 31	501 ± 34	ns
Spike biomass (142 DAS) (g m^{-2})	**104 ± 13**	**153 ± 10**	*****
Leaf biomass (142 DAS) (g m^{-2})	75.6 ± 8.1	96.5 ± 7.1	ns
Stem biomass (142 DAS) (g m^{-2})	197 ± 16	251 ± 17	ns
RGR_{shoot} (142 DAS) (mg g^{-1} day^{-1})	25.6 ± 1.2	28.6 ± 0.8	ns
Flag-leaf area (cm^2)	**24.1 ± 0.7**	**33.8 ± 1.6**	******
$SLA_{Flag\text{-}leaf}$ (m^2 kg^{-1})	21.6 ± 0.3	19.4 ± 0.6	ns
Spike biomass (173 DAS) (g m^{-2})	602 ± 25	656 ± 25	ns
Grain production (g m^{-2})	451 ± 17	497 ± 14	ns
Spike biomass fraction (173 DAS) (g g^{-1})	**0.55 ± 0.01**	**0.61 ± 0.01**	*****

DAS days after sowing. *SLA* specific leaf area

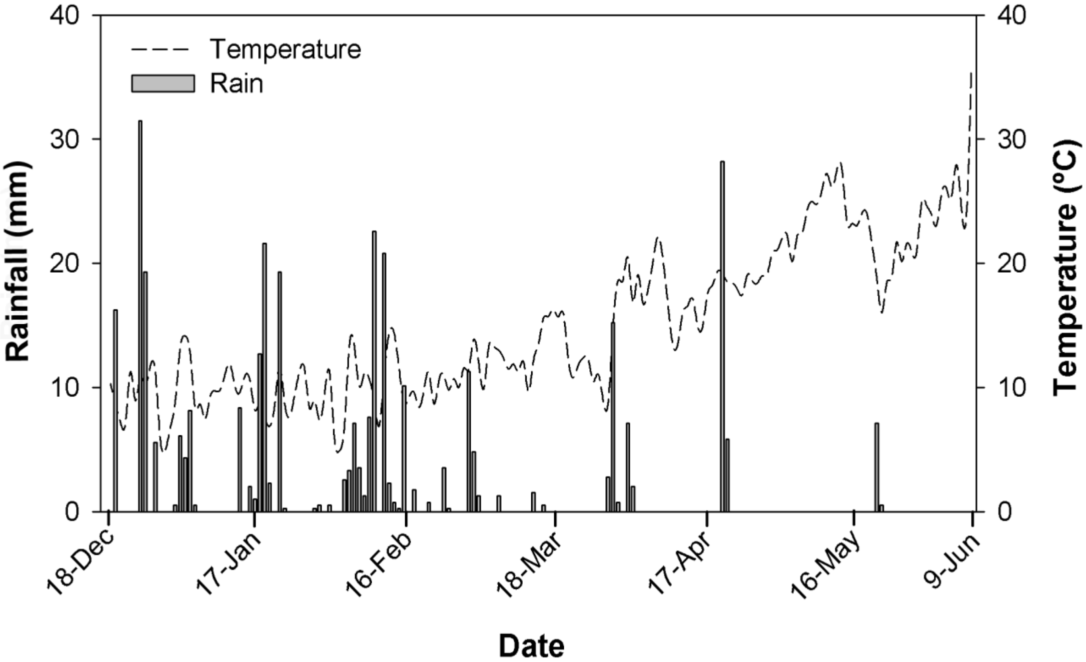

Fig. S1 Mean air temperature and rainfall during the wheat crop season

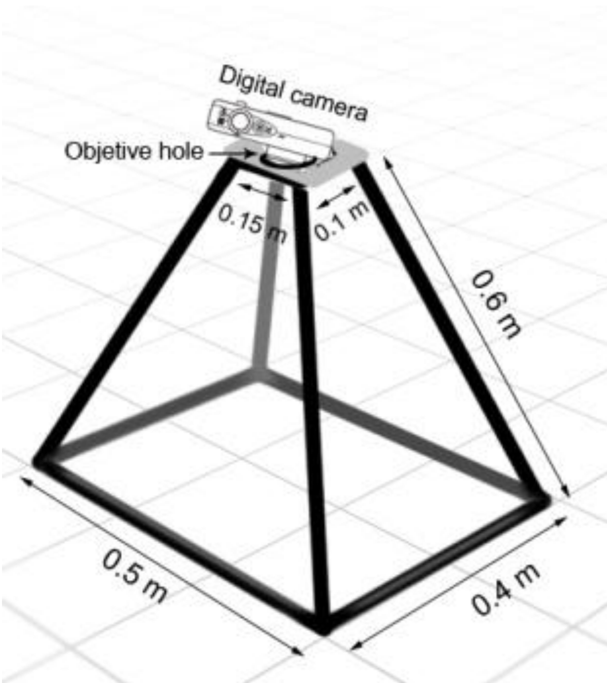

Fig. S2 Model of the tripod used for the visual categorization of biochar content of soil. It was made with bamboo sticks attached with tape

Capítulo 6. Discusión general

El objetivo principal de esta tesis doctoral es evaluar los efectos del biochar sobre las propiedades del suelo y cómo estos cambios influyen en el desarrollo y crecimiento de las plantas. Para ello, se han combinado ensayos en condiciones controladas (cámara de cultivo e invernadero) con ensayos en campo y se ha profundizando en el estudio de aquellos factores que van a determinar la disponibilidad de recursos (agua y nutrientes) para las plantas.

Las respuestas de los rasgos funcionales de la planta a la adición de biochar constituyen un reflejo de la disponibilidad de estos recursos. Por tanto, su estudio aporta un conocimiento más completo acerca de los mecanismos específicos de las plantas relacionados con las respuestas de su crecimiento a la adición de biochar al suelo.

La Fig. 1 muestra un esquema general con los principales resultados obtenidos de esta tesis doctoral. A continuación, se presenta una discusión general de los mismos.

La adición de biochar modificó las características del suelo

Entre los resultados más importantes que se extraen de forma consistente de esta tesis doctoral están la reducción de la densidad aparente y la compactación del suelo con la adición de biochar. La reducción de la densidad aparente se ha asociado con la elevada porosidad y la baja densidad del biochar (Oguntunde et al. 2008; Laird et al. 2010a). Estas características también explicarían la reducción de la compactación que observamos en los experimentos de campo (capítulos 4 y 5), similar a la encontrada en otros estudios (Busscher et al. 2010; Mukherjee y Lal 2013).

Desde un punto de vista agronómico, la reducción de la densidad aparente y de la compactación favorece la aireación del suelo y aumenta la capacidad de infiltración de agua, lo que beneficia al desarrollo de la raíz y de los microorganismos del suelo (Alameda et al. 2009; Downie et al. 2009; Laird et al. 2010a). Este hecho puede resultar decisivo en suelos que por sus características físicas y propiedades mecánicas favorezcan su compactación, constituyendo un factor limitante para el desarrollo de los cultivos.

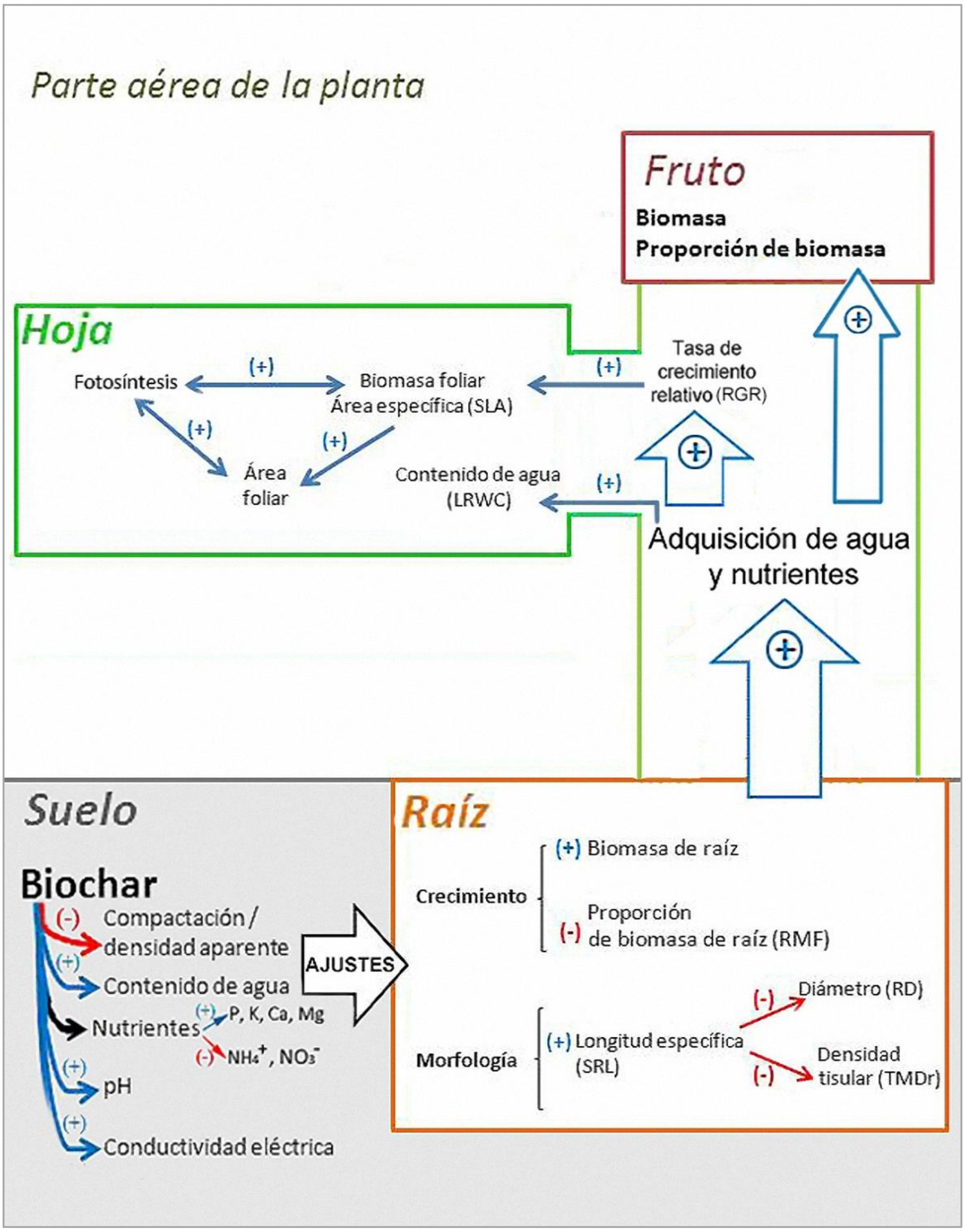

Figura 1. Resumen de los principales resultados de esta tesis doctoral sobre los efectos de la adición de biochar al suelo en relación a los cambios en las características del suelo y los mecanismos de respuesta de las plantas.

Cabe recordar que los experimentos de campo se desarrollaron sobre suelos arcillosos, tipo Vertisol, cuyas propiedades físicas y régimen de humedad presentan serias limitaciones. En ellos domina la presencia de arcillas expandibles, otorgándoles una textura pesada y un estrecho margen entre el estrés hídrico y el exceso de agua. El encharcamiento es un factor importante, ya que reduce el periodo para el desarrollo vegetal. Por tanto, cualquier mejora de las propiedades físicas de estos suelos, como la aportada por la adición de biochar, puede influir muy positivamente sobre la producción del cultivo como se puso de manifiesto en los capítulos 4 y 5.

Otra característica importante del suelo y relacionada a su vez con la densidad aparente y la compactación, es la capacidad de retención hídrica del suelo (Ueckert et al. 1978; Alameda et al. 2012). En general, encontramos un aumento del contenido de agua del suelo con la adición de biochar. Así por ejemplo, la adición de biochar en maceta aumentó el contenido de agua de suelos arenosos (capítulos 2 y 3) y un efecto similar fue obtenido en campo (capítulos 4 y 5). Nuestros resultados concuerdan con los obtenidos en otros estudios, que asociaron el aumento del contenido de agua del suelo con la retención física de agua en los poros del biochar y a la presencia de grupos funcionales hidrofílicos en la superficie del biochar (Chan et al. 2007; Laird et al. 2010b; Basso et al. 2013). Como consecuencia, la enmienda de suelo con biochar puede aumentar la capacidad de retención de agua, lo que podría favorecer la producción de los cultivos en zonas semiáridas y reducir las necesidades de agua en cultivos de regadío. Además, el aumento de la capacidad de retención hídrica puede ser un factor muy importante en suelos arenosos, caracterizados por una elevada permeabilidad, permitiendo retener mayor cantidad de agua disponible para las plantas durante periodos de tiempo más largos.

El biochar aumentó el pH del suelo, especialmente cuando éste se aplicó a altas dosis (capítulos 2, 3 y 5). Estos resultados fueron consistentes con las propiedades de los biochars utilizados en estos ensayos y también concuerdan con los resultados de otros estudios (Atkinson et al. 2010; Van Zwieten et al. 2010; Yuan y Xu 2011). En suelos ácidos, el aumento del pH puede mejorar la calidad del suelo, aumentando la disponibilidad de nutrientes y reduciendo la disponibilidad de elementos perjudiciales para la planta como por ejemplo Al (Qian et al. 2013). Se considera que los biochars con un pH > 9 tienen un efecto de encalado del suelo (Van Zwieten et al. 2010). Por otra parte, la adición de biochar a suelos alcalinos no modifica en gran medida su pH, como encontramos en los capítulos 4 y 5, este hecho se justificó por el alto contenido del suelo en carbonatos y su fuerte poder tamponante frente a cambios de pH. Cabe señalar que en suelos con pH por encima de la neutralidad es importante comprobar que la adición de biochar no agrave problemas asociados a deficiencias de micronutrientes (Chan y Xu 2009). El biochar también aumentó la conductividad eléctrica del suelo debido a la presencia de sales solubles en este material, lo que concuerda con los resultados de otros estudios (Chan et al. 2008; Méndez et al. 2012; Chintala et al. 2014).

Las propiedades físicas y químicas del biochar son claves para entender los principales efectos del biochar en el suelo. El empleo de diferentes tipos de biochars y dosis de aplicación bajo diferentes condiciones experimentales nos ha proporcionado una perspectiva más completa acerca de los cambios más significativos que el biochar provoca sobre las características físicas y químicas del suelo que incluyeron la modificación de la estructura del suelo (reducción de la densidad aparente y la compactación) y el aumento de la capacidad de retención hídrica, el pH y la conductividad eléctrica.

La adición de biochar alteró la disponibilidad de nutrientes del suelo

La adición de biochar alteró la disponibilidad de nutrientes por medio de dos mecanismos fundamentalmente, mediante su aporte directo y su retención. Tanto en los ensayos en maceta como en los de campo, encontramos un aumento generalizado del contenido y disponibilidad de algunos nutrientes del suelo (P, K, Ca, Mg, Cu y Zn). Este aumento puede justificarse por el aporte directo de estos nutrientes con el propio biochar en formas fácilmente disponibles (Chan y Xu 2009; Tammeorg et al. 2014).

Por otra parte, las características propias del biochar, (elevada porosidad, superficie específica y capacidad de intercambio iónico) favorecen la retención de nutrientes que de otro modo podrían perderse por lavado, ejerciendo un impacto positivo sobre la disponibilidad de nutrientes a largo plazo (Cheng et al.

2006; Ippolito et al. 2015). Sin embargo, la capacidad del biochar para retener nutrientes también puede limitar su disponibilidad a corto plazo como quedó demostrado en el capítulo 2, donde encontramos una reducción de los contenidos de amonio y nitrato extraíble del suelo a medida que aumentaba la dosis de biochar. Estos descensos se asociaron principalmente a la alta capacidad de retención de estas formas de N por el biochar (Lehmann et al. 2003; Atkinson et al. 2010). Además, en ese mismo capítulo encontramos una reducción de la disponibilidad de Fe y Mn como consecuencia del efecto de encalado del biochar. A pesar de ello, no se detectaron síntomas de deficiencia en las plantas y la adición de biochar aumentó la producción de grano (especialmente cuando se combinó con una fertilización).

Como contrapunto a lo anterior, en todos los ensayos encontramos un aumento del contenido y disponibilidad de P en el suelo, asociado principalmente a su aporte directo con el biochar. Este aumento de la disponibilidad de P benefició a los cultivos ensayados en nuestro estudio y puede suponer un factor decisivo, ya que los suelos de nuestra región suelen ser deficientes en P (Solis y Torrent 1989). Otros estudios han encontrado que los cambios en el pH del suelo y el aumento de la actividad microbiana tras la adición de biochar aumentaron la disponibilidad de P (Atkinson et al. 2010; Anderson et al. 2011; Xu et al. 2014), por tanto estos factores no pueden ser descartados en nuestro estudio.

Otro resultado interesante fue que el biochar mejoró la eficiencia del fertilizante añadido (capítulo 2). Así, el rendimiento de cultivo obtenido cuando el biochar se aplicó de forma combinada con una fertilización fue siempre superior al obtenido sólo con la fertilización, resultados que concuerdan con estudios previos (Atkinson et al. 2010; Chan et al. 2007; Zheng et al. 2013).

Nuestros resultados demuestran la complejidad del estudio de las interacciones nutriente-biochar, ya que son altamente dependientes del nutriente involucrado, el tipo de biochar aplicado y las características del suelo. Como ha quedado demostrado, el beneficio agronómico del biochar va a depender en gran medida de su impacto sobre la disponibilidad de nutrientes. El aumento que hemos encontrado en el contenido disponible de nutrientes y en la eficiencia del fertilizante tras la adición de biochar puede promover una agricultura más sostenible a largo plazo. El uso de fertilizantes supone una gran inversión económica por parte de los agricultores, de modo que, el aumento de su eficiencia con la adición de biochar podría reducir la tasa de aplicación de fertilizantes, disminuyendo el coste y los impactos negativos medioambientales derivados del uso excesivo de fertilizantes. En este sentido, es de suma importancia evaluar qué mecanismos gobiernan los fenómenos de adsorción, desorción y precipitación que van a determinar en gran medida que los nutrientes retenidos por el biochar sean más o menos disponibles para la planta.

Los cambios en las características del suelo explican las respuestas de la raíz a la adición de biochar

El estudio de las interacciones entre la raíz y el biochar nos ha proporcionado una información más completa acerca de los efectos del biochar sobre el suelo (variables físicas y disponibilidad de nutrientes) y de sus implicaciones sobre el desarrollo de las plantas. Nuestros resultados muestran que la adición de biochar afectó a las características de la raíz, aunque la magnitud de sus respuestas dependió de las condiciones experimentales de cada ensayo.

Una de las respuestas más comunes de la planta a la adición de biochar fue la reducción de la proporción de la biomasa de raíz (RMF). Sin embargo, el biochar no redujo la biomasa de raíz en términos absolutos (excepto el biochar de paja de trigo, que en el capítulo 2 redujo la biomasa de raíz en plantas de trigo). Los resultados sugieren que la reducción de RMF se debió al aumento considerable del crecimiento de la parte aérea (hojas, tallos y frutos). Este tipo de respuestas se han asociado a condiciones de alta disponibilidad de recursos en el suelo (Müller et al. 2000; Shipley y Mezaine 2002), lo que concuerda con el aumento generalizado del contenido de agua y nutrientes que obtuvimos tras la adición de biochar. De acuerdo con nuestros resultados, Biederman y Harpole (2013) también encontraron un aumento de la biomasa de la parte aérea con la adición de biochar, sin embargo, no encontraron un efecto claro del biochar sobre RMF.

En general, el biochar también afectó a la morfología de la raíz, aumentando la longitud específica de la raíz (SRL) tanto en ensayos en maceta (capítulos 2 y 3) como en campo (capítulo 4), aunque la magnitud de sus efectos también dependió de las condiciones particulares de cada estudio. Considerando los resultados en las distintas especies, encontramos que seis de las nueve especies estudiadas aumentaron SRL con la adición de biochar. Al aumentar SRL aumenta la longitud de raíz por unidad de biomasa de raíz y el volumen de suelo explorado por unidad de biomasa de raíz invertida, mejorando la eficiencia de la raíz para captar recursos (Eissenstat 1992; Ryser 2006; Lambers et al. 2006).

Los cambios en la morfología de la raíz de trigo (capítulo 2) estuvieron relacionados con los cambios en la disponibilidad de los nutrientes provocados por la adición de biochar. En concreto, el biochar redujo la disponibilidad de nitrato y amonio que se correlacionó con un aumento de SRL y una reducción del diámetro de la raíz (RD). Es de suponer que al aumentar del SRL (mayor proliferación de la raíz) aumentaron las interacciones raíz-biochar y por consiguiente el acceso de la raíz a los nutrientes retenidos en el biochar. Además, estos ajustes en la morfología de la raíz podrían explicar el efecto positivo de la mezcla biochar-fertilizante, como demostró el hecho de que la producción de las plantas tratadas con una combinación de biochar-fertilizante fuese mayor que las de las plantas tratadas exclusivamente con fertilizante (capítulo 2).

Cabe esperar que los ajustes morfológicos de la raíz tras la enmienda de suelo con biochar afecten a la adquisición de los recursos y sean determinantes para la producción de la planta. En este sentido, encontramos una relación positiva y significativa entre el aumento de la producción por efecto del biochar y el aumento de SRL (capítulo 3), lo que indica que las especies que más aumentaron la longitud específica de la raíz (SRL) tras adicionar biochar al suelo son las que más aumentaron su producción. En el capítulo 4 también encontramos que la producción de trigo en condiciones de campo estuvo positivamente relacionada con un aumento en SRL. El aumento de SRL se explicó en mayor medida por la reducción de la densidad tisular de la raíz (TMDr; en capítulos 3 y 4) y en menor medida por la reducción del diámetro de la raíz (RD; en capítulo 2). La reducción de TMDr (relación biomasa/volumen de la raíz) implica una reducción de la biomasa de raíz por volumen de raíz, lo que se traduce en una reducción del coste de construcción de raíz (Eissenstat 1992). Estos resultados concuerdan con Prendergast-Miller et al. (2014), quienes encontraron una mayor proliferación de raíz fina en cebada en los suelos tratados con biochar. Así, los ajustes en la morfología de la raíz pueden explicarse por los cambios inducidos por el biochar sobre las características del suelo. Estos cambios en la raíz favorecieron un mayor acceso de la misma a los recursos del suelo y del biochar, y por tanto, promovieron el desarrollo, crecimiento y producción de las plantas.

La adición de biochar aumentó el crecimiento y la producción vegetal

En el capítulo 3 encontramos que, por lo general, las especies del estudio respondieron a la adición de biochar produciendo hojas con mayor área específica foliar (SLA) y biomasa de hojas, lo que se tradujo en una mayor superficie fotosintética (Poorter y Evans 1998; Reich et al. 1998). Además, las plantas tratadas con biochar presentaron un estado hídrico más elevado. Como resultado, el biochar de poda de olivo promovió generalmente el crecimiento de las ocho especies estudiadas, lo que indica su idoneidad como enmienda para aumentar la producción de un amplio rango de especies. Estos resultados concuerdan con los aumentos en la producción obtenidos en otros estudios (Chan et al. 2007; Van Zwieten et al. 2010). Nuestros experimentos en campo también confirmaron estos resultados ya que el biochar de poda de olivo aumentó la tasa de crecimiento relativo (RGR) del trigo, lo que pudo estimular su producción (Araus et al. 1986; Araus y Tapia 1987).

Así mismo, encontramos que el biochar aumentó la producción de trigo alrededor de un 20 % (capítulos 4 y 5). Otros estudios en campo han encontrado aumentos en la producción de trigo similares a los nuestros (Baronti et al. 2010; Vaccari et al. 2011; Karer et al. 2013). Liu et al. (2013) en un meta-análisis (con 103 estudios) analizaron los efectos del biochar sobre la producción vegetal, encontrando un aumento medio del 11 % en la producción, muy similar a los resultados de otro meta-análisis realizado por Jeffery et al. (2011), con un aumento medio del 10 %. Sin embargo, estas revisiones muestran una amplia variación en los resultados dependiendo de la especie de estudio, con rendimientos para algunos casos similares a los nuestros.

Los mecanismos que se han propuesto para explicar el aumento de la producción han incluido cambios en el pH del suelo y el aumento de la disponibilidad agua y de nutrientes tras la adición de biochar (Vaccari et al.

2011; Baronti et al. 2014; Tammeorg et al. 2014). A menudo resulta complicado determinar qué factor o factores son los más importantes para justificar el aumento de la producción de los cultivos. Nuestros resultados sugieren que la reducción de la compactación y el aumento del contenido de agua del suelo fueron los principales factores que explicaron el aumento de la producción en los ensayos en campo (capítulos 4 y 5) ya que los suelos presentaban una fertilidad media/alta. La disponibilidad de agua suele ser un factor limitante para la producción de los cultivos de secano en el área mediterránea (Bennet et al. 1998). Sin embargo, el aumento de la disponibilidad de nutrientes tras la adición de biochar en los ensayos en maceta con suelos relativamente pobres en nutrientes pudo ser más relevante que los cambios en las propiedades físicas, ya que las macetas se regaban con frecuencia y los suelos eran de textura arenosa y por tanto, con bajas limitaciones para el desarrollo de la raíz.

El manejo del suelo alteró la distribución del contenido de biochar aplicado en campo

Una vez que el biochar se ha distribuido e incorporado de forma homogénea en el suelo, los movimientos posteriores del mismo con el arado y la siembra pueden modificar su distribución espacial inicial y alterar su contenido en el suelo (Leifeld et al. 2007). Este efecto se ve favorecido por la propia heterogeneidad que presenta el biochar. En función del material empleado para su producción, el biochar resultante puede estar constituido por fracciones con un rango amplio de tamaño de partícula, desde fracciones muy finas hasta fracciones medias y gruesas de varios centímetros de longitud. A modo de ejemplo, basta comparar un material muy homogéneo de partida como es el hueso de aceituna con una poda de olivo, que incluso triturada puede presentar fracciones de gran tamaño. Si tenemos en cuenta que el biochar no va a ser aplicado de forma continua año tras año (su naturaleza recalcitrante favorece un efecto residual a largo plazo), es de suma importancia que el biochar se incorpore y distribuya de forma correcta en el suelo y que ésta se mantenga en el tiempo.

En el capítulo 5 evaluamos la heterogeneidad de la distribución del contenido de biochar en el suelo un año después de su aplicación en campo. Para determinar el contenido de biochar en el suelo usamos una caracterización visual "in situ" basada en la tonalidad negra que el biochar confiere al suelo. La Fig. 2 muestra un esquema general de los principales efectos de la heterogeneidad en la distribución del contenido de biochar sobre las características del suelo y sobre el cultivo. Nuestros resultados mostraron que la estimación visual del contenido de biochar en el suelo estuvo correlacionada positiva y significativamente tanto con el contenido de biochar como con el color del suelo (determinados por calcinación y por espectroscopia de reflectancia difusa, respectivamente), lo que indica que la categorización visual constituye un método rápido y económico para determinar el contenido de biochar en el suelo. Los resultados indicaron también que las parcelas tratadas inicialmente a una concentración homogénea de biochar (4 kg m^{-2}) presentaron un año después una distribución heterogénea del mismo, mostrando zonas del suelo con un mayor contenido en biochar y zonas con un menor contenido.

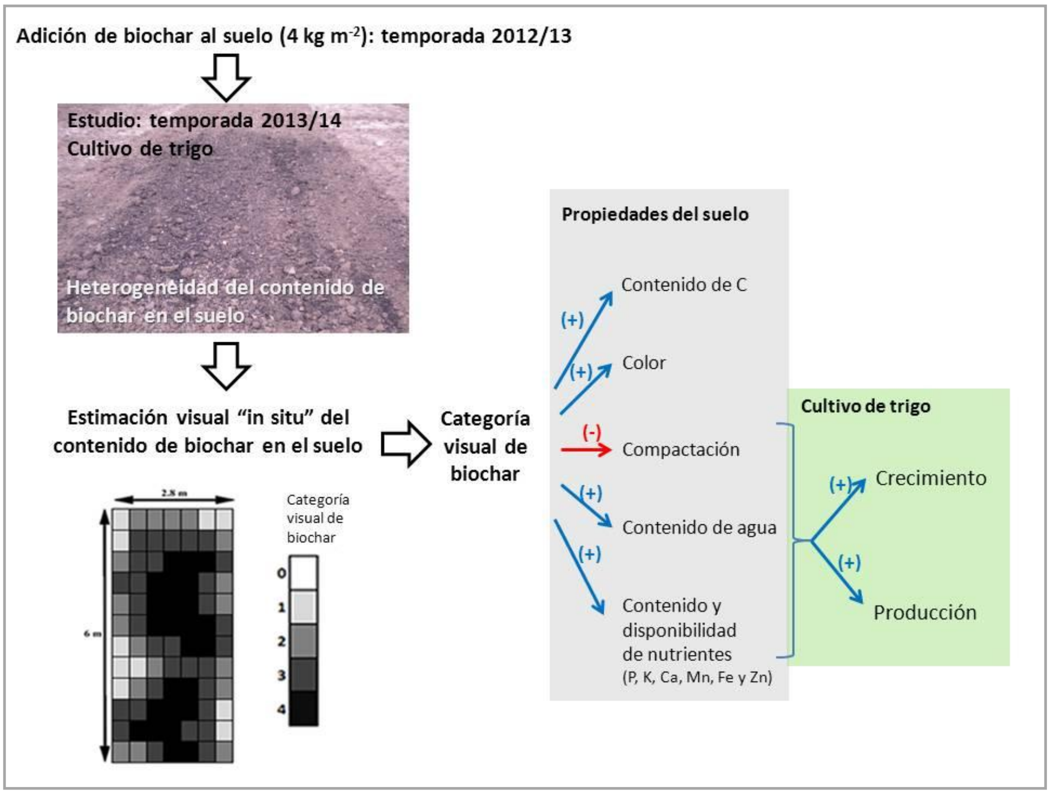

Figura 2. Principales efectos de la heterogeneidad en la distribución del contenido de biochar sobre las características del suelo y sobre el cultivo.

La heterogeneidad detectada en el contenido de biochar del suelo afectó a las propiedades del suelo. Así, las zonas enriquecidas en biochar reforzaron el efecto del biochar, la compactación del suelo disminuyó mientras que la capacidad de retención hídrica y el contenido de nutrientes del suelo aumentaron, lo que aumentó el crecimiento y la producción del cultivo. Sin embargo, en las zonas con un menor contenido de biochar su efecto beneficioso sobre el cultivo fue menor. Por tanto, al analizar los datos sin tener en cuenta la heterogeneidad, es decir, considerando solamente los dos tratamientos aplicados inicialmente (control y biochar), no obtuvimos efectos positivos del biochar sobre el cultivo. Estos resultados subrayan la importancia de considerar la heterogeneidad en ensayos de campo, ya que podría ser otro factor que explicase la ausencia de efectos significativos del biochar sobre el cultivo en los años posteriores a la adición de biochar.

Se debe prestar especial atención en aquellos suelos que presenten dificultades de manejo y laboreo debido a propiedades tales como textura pesada, fácil compactación, etc. En estos casos se recomienda el uso de biochars lo más homogéneos posibles y de pequeño tamaño de partícula que favorezca su rápida y fácil incorporación en el suelo. Si el biochar no cumple con estos requerimientos siempre se pueden aplicar pre- y post-tratamientos al biochar (trituración, clasificación, peletización, etc.), asumiendo que con ello se incrementarían los costes de producción.

En resumen, los resultados sugieren que el biochar puede ser usado para mejorar la calidad del suelo, aumentar la eficiencia de los fertilizantes y la producción de un amplio rango de especies agronómicas.

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Conclusiones

Las siguientes conclusiones constituyen una síntesis de los resultados más relevantes expuestos en la presente tesis doctoral:

1. La adición de biochar aumentó la capacidad de retención hídrica del suelo, y redujo su densidad aparente y compactación.

2. El biochar aumentó el pH, en menor medida en suelos de pH alcalino, y la conductividad eléctrica del suelo.

3. El efecto del biochar sobre la disponibilidad de nutrientes del suelo dependió del nutriente involucrado, por una parte aumentó el contenido de algunos (Ca, K, Mg, P, Cu y Zn) y por otra parte retuvo o inmovilizó otros (amonio, nitrato, Fe y Mn), reduciendo su disponibilidad para la planta. Estos efectos dependieron en gran medida de las características del suelo y de las condiciones de fertilización.

4. Los cambios inducidos por el biochar en la disponibilidad de los nutrientes del suelo fueron claves para explicar los efectos del biochar sobre la raíz. El biochar aumentó la longitud específica de la raíz, haciendo las raíces más finas y con tejido menos denso.

5. El aumento de la longitud específica de la raíz tuvo implicaciones sobre el desarrollo de la planta en su conjunto, y promovió el crecimiento vegetal y la producción de frutos al facilitar un mayor acceso a los recursos (agua y nutrientes) presentes en el suelo y especialmente en el biochar.

6. El manejo del suelo alteró la distribución espacial del contenido de biochar que había sido aplicado un año antes en campo, lo que conllevo que éste mostrase una elevada heterogeneidad. La mayoría de los estudios en campo a corto y medio plazo no consideran la movilización del biochar, lo que puede dar lugar a interpretaciones erróneas cuando se relacionan las respuestas del cultivo asociadas a la adición de biochar.

7. Nuestros resultados indican que el biochar puede ser una solución viable para mejorar las propiedades físico-químicas del suelo y aumentar el crecimiento y la producción de los cultivos. En este sentido, cabe señalar como aspectos clave para asegurar la eficacia agronómica del biochar: *i*) una buena selección del biochar que se emplea, adecuando sus características a las propiedades del suelo y condiciones de cultivo; y *ii*) un estudio de las

interacciones nutriente-biochar, determinando qué factores condicionan la disponibilidad de los nutrientes retenidos en el biochar.

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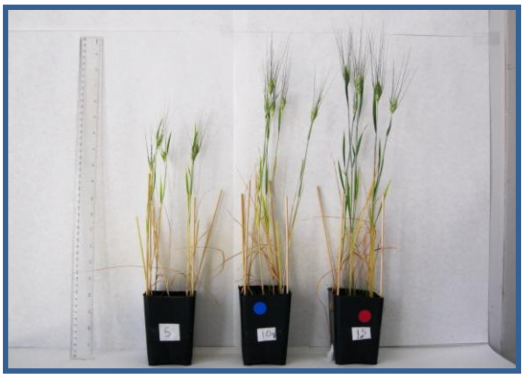
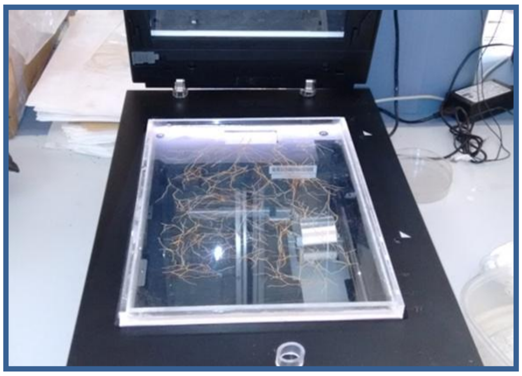
Anexo fotográfico

Capítulo 2. Changes in soil nutrient availability explain biochar's impact on wheat root development

Experimento en cámara de cultivo. Plantas de trigo en maceta y resinas de intercambio iónico.



Plantas tratadas con biochar y tres niveles de fertilización (de izquierda a derecha: control, medio y alto).



Escáner de doble lámpara usado para analizar la morfología de la raíz.

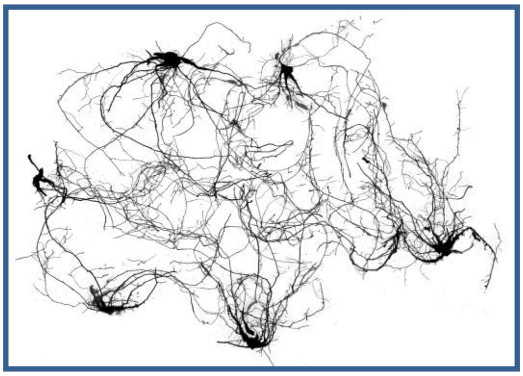


Imagen obtenida a partir del escaneo de la raíz.

Capítulo 3. Biochar affects fruit production in eight agronomic species through changes in root traits

Raíz de pimiento limpia.

Algunas de las especies utilizadas en el experimento (de izquierda a derecha: tomate, pimiento y soja).

Capítulo 4. Wheat growth and yield responses to biochar addition under Mediterranean climate conditions

Adición de biochar de poda de olivo en campo.

Parcela tratada con biochar.

Extracción de la raíz de trigo en campo con una sonda cilíndrica.

Estado del cultivo en el momento de la cosecha.

Capítulo 5. Spatial heterogeneity of soil biochar content affects soil quality and wheat growth and production

Distribución espacial del contenido de biochar en el suelo un año después de su adición.

Soporte fabricado para tomar las fotografías del suelo usadas para la estimación visual del contenido de biochar.

Aportaciones divulgativas

Noticia publicada en el periódico *ABC*

¿Es rentable su fabricación?

El profesor Villar asegura que actualmente la poda del olivo se tritura y se devuelve al campo. Como consecuencia, se descompone y el CO2 se libera a la atmósfera. «Y si hay enfermedades, al molerlo se pueden expandir por el suelo. Con el biocarbón se ahorraría la emisión de CO2 y la difusión de plagas, además de mejorar la cosecha». El reto es conseguir que su producción sea económicamente viable. «Ahora hay empresas que trabajan ya en el desarrollo de maquinaria capaz de triturar y priolizar in situ el material de poda o desechos». Por ahora el biocarbón es un material caro pero Villar avanza que «será usado a medio plazo cuando se desarrolle la maquinaria para hacerlo a menor coste».

Se cree que el aumento de la producción está relacionado con el uso de este producto vegetal ABC

Científicos andaluces estudian su capacidad para aumentar la fertilidad del suelo y mejorar las cosechas

Biocarbón, *el abono verde* que combate el cambio climático

E. NAVAS SEVILLA

El biocarbón, también llamado biochar, es un producto similar al carbón que se produce por el calentamiento de materia vegetal en una atmósfera pobre en oxígeno. Por este procedimiento, denominado pirolisis lenta, alrededor de la mitad del carbono de la biomasa queda almacenado en el biocarbón, de ahí que resulte un material beneficioso como sumidero de CO2 que contribuye a reducir el efecto invernadero.

Sin embargo su uso no es nuevo. Según un estudio de los suelos amazónicos, conocidos como «Terra preta», revela que es un material muy estable que puede permanecer en el suelo entre 500 y 7.000 años. Además se trata de terrenos múy fértiles que sugieren que el biocarbón vendría a mejorar la producción agrícola.

Debido a estas y otras cualidades, los científicos han comenzado a estudiar las caracteristicas del biochar procedentes de distintos residuos vegetales y sus efectos sobre las propiedades del suelo y el crecimiento de las cosechas. Las investigaciones se enmarcan en el Proyecto Biocar, (financiado por el Ministerio de Economia y Fondos Feder) y en las que han participado científicos de las universidades de Córdoba, de la Pablo de Olavide de Sevilla y de la empresa Abengoa. Dos recientes trabajos publicados por investigadores de la Universidad cordobesa, desvelan algunas de sus características.

En el primer trabajo, publicado en la revista Biology and Fertility of Soils, los científicos cordobeses analizaron el efecto del biocarbón producido a partir de restos de poda del olivo sobre el cultivo del trigo. Para ello, dividieron una parcela experimental en bloques. A la mitad se les añadió biocarbón en una cantidad de 4 kilos por metro cuadrado, mientras que el otro 50% no recibió tratamiento.

Los resultados revelaron que la adición del biocarbón aumentó la capacidad de retención de agua del suelo y redujo su grado de compactación. «La mejora de las propiedades físicas del suelo puede tener un papel decisivo en climas secos, como el mediterráneo, en el que la baja disponibilidad de agua es un factor limitante para la agricultura», explican sus autores.

Además se pudo comprobar que los suelos que recibieron este producto también aumentaron su contenido en nutrientes. «Vimos que el biocarbón actúa como si fuera una esponja que retiene los nutrientes», explica Rafael Villar, profesor de Ecología de la Universidad de Córdoba y uno de los autores del estudio. Asimismo añade que, aunque no lo observaron directamente, «parece que las plantas desarrollan una mayor proporción de raíces finas que envuelven al biochar».

Esta misma fuente señala que las raíces finas «hacen que la planta asimile mejor los nutrientes y el agua, y ello desemboca en un mejor desarrollo de la planta». A su juicio, todos estos cambios han podido ser responsables del aumento en un 27% de la producción de trigo en las parcelas tratadas con este producto vegetal.

En un segundo trabajo, publicado en la revista Journal of Plant Nutrition and Soil Science, los autores evaluaron los efectos del biocarbón procedente de diferentes orígenes, tales como huesos de aceitunas, cáscaras de almendras, paja de trigo, astillas de madera de pino y poda de olivos, sobre plantas de girasol cultivadas en un invernadero experimental, en el que se controlaron las condiciones ambientales. Los resultados evidenciaron que el efecto del biocarbón es diferente según su origen. Algunos son muy porosos, como los que provienen de la paja de trigo, mientras que otros como el de hueso de aceituna son más densos y ello tiene consecuencias sobre la densidad de los suelos.

De todo ello se deriva que, si bien el biochar tiene mucho potencial para mejorar la productividad de los suelos agrícolas «su uso debe basarse en las propiedades específicas de cada biocarbón, prestando especial atención a su efecto sobre la disponibilidad de nutrientes en el suelo», explican los autores del trabajo.

Factor
La mejora de las propiedades del suelo puede tener un papel decisivo en climas secos

Audio entrevista en la web *www.cienciaes.com*

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Biocarbón. Hablamos con Rafael Villar.

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El pasado y el presente de la humanidad se apoyan en infinidad de pequeños pasos, gracias a los cuales hemos podido alcanzar un desarrollo tecnológico y social sin precedentes en la historia. El progreso de nuestra especie nos ha permitido sobrevivir y prosperar de tal manera que la humanidad ha dejado de ser insignificante frente al tamaño de nuestro planeta y como consecuencia, nuestras acciones también han adquirido dimensiones planetarias.

El desarrollo, basado fundamentalmente en el consumo de combustibles fósiles, está liberando a la atmósfera una cantidad enorme de gases de efecto invernadero que están provocando la elevación de la temperatura media del planeta y un cambio climático de consecuencias imprevisibles. El dióxido de carbono liberado a la atmósfera continúa creciendo cada día y el cambio climático ya ha dejado de ser una hipótesis incómoda para convertirse en una realidad amenazadora.

Manuel Olmo — Dr. Rafael Villar — Dr. José A. Alburquerque
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El Día de Córdoba, Noticias de Córdoba y su Provincia > Córdoba > Estudian usar el biocarbón como abono contra el cambio climático

Estudian usar el biocarbón como abono contra el cambio climático

EL DÍA | ACTUALIZADO 14.05.2015 - 01:00

Investigadores de la Universidad de Córdoba (UCO) estudian las posibilidades del biocarbón como el mejor "abono verde" para combatir y mitigar los efectos del cambio climático, mejorar la producción de los cultivos y dar salida a residuos vegetales como los procedentes de la poda del olivo. La institución académica informó ayer de que el biocarbón o *biochar* es "un producto similar al carbón que se produce por el calentamiento de materia vegetal en una atmósfera pobre en oxígeno".

Por este procedimiento, denominado pirolisis lenta, alrededor del 50% del carbono de la biomasa queda almacenado en el biocarbón, por lo que resulta un material muy interesante como sumidero de CO_2 que contribuya a reducir el efecto invernadero. Según la misma información, el uso del biocarbón no es nuevo, y el estudio de los suelos amazónicos conocidos como *Terra Preta* revela que es un material muy estable que puede permanecer en el suelo entre 500 y 7000 años. La UCO destacó que además, se trata de suelos muy fértiles, que sugieren que el biocarbón podría mejorar la producción agrícola, por lo que los científicos están empezando a estudiar las características del biocarbón procedente de distintos residuos vegetales y sus efectos sobre las propiedades del suelo y el crecimiento de las cosechas.