DISIPACIÓN DE HERBICIDAS EN UN SUELO ENMENDADO CON RESIDUOS ORGÁNICOS: ENSAYOS EN CAMPO E IMPACTO EN LAS COMUNIDADES MICROBIANAS DEL SUELO

Víctor Barba Vicente

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Memoria presentada por el Licenciado Víctor Barba Vicente para optar al grado de Doctor por la Universidad de Salamanca dentro del programa de doctorado de Agrobiotecnología.

Salamanca, 24 de octubre de 2019

Víctor Barba Vicente

Dña. María Jesús Sánchez Martín, Profesora de Investigación del CSIC, Dña. María Sonia Rodríguez Cruz, Científica Titular del CSIC y D. Jesús María Marín Benito, Investigador Postdoctoral Contratado del CSIC, del Instituto de Recursos Naturales y Agrobiología de Salamanca, directores del trabajo **"Disipación de herbicidas en un suelo enmendado con residuos orgánicos: ensayos en campo e impacto en las comunidades microbianas del suelo"** realizado por el licenciado Víctor Barba Vicente en los laboratorios del Instituto de Recursos Naturales y Agrobiología de Salamanca (IRNASA-CSIC) para optar al grado de Doctor por la Universidad de Salamanca, autorizan la presentación del mismo en el formato de compendio de artículos al considerar que se han alcanzado los objetivos inicialmente previstos.

Y para que así conste, firman el presente certificado

Salamanca, 24 de octubre de 2019

Fdo.: María Jesús Sánchez Martín

Fdo.: María Sonia Rodríguez Cruz

Fdo.: Jesús María Marín Benito

El presente trabajo **"Disipación de herbicidas en un suelo enmendado con residuos orgánicos: ensayos en campo e impacto en las comunidades microbianas del suelo"** ha sido realizado en el Departamento de Procesos de Degradación del Medio Ambiente y su Recuperación del Instituto de Recursos Naturales y Agrobiología de Salamanca (IRNASA-CSIC) bajo la dirección de la Prof. Dra. Mª Jesús Sánchez Martín, la Dra. Mª Sonia Rodríguez Cruz y el Dr. Jesús María Marín Benito.

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1. "Application of green compost as amendment in an agricultural soil: Effect on the behaviour of triasulfuron and prosulfocarb under field conditions"

   J.M. Marín-Benito[a], V. Barba[a], J.M. Ordax[a], M.S. Andrades[b], M.J. Sánchez-Martín[a], M.S. Rodríguez-Cruz[a]

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   Journal of Environmental Management 207 (2018), 180-191

2. "Simultaneous application of two herbicides and green compost in a field experiment: Implications on soil microbial community"

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   Applied Soil Ecology 127 (2018), 30–40

3. "Recycling organic residues in soils as amendments: Effect on the mobility of two herbicides under different management practices"

   J.M. Marín-Benito, V. Barba, J.M. Ordax, M.J. Sánchez-Martín, M.S. Rodríguez-Cruz

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   Journal of Environmental Management 224 (2018), 172–181

4. "Influence of different agricultural management practices on soil microbial community over dissipation time of two herbicides"

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   Science of the Total Environment 646 (2019), 1478–1488

5. “Assessment of ^{14}C-prosulfocarb dissipation mechanism in soil after amendment and its impact on the microbial community”

   Víctor Barba, Jesús M. Marín-Benito, Carlos García-Delgado, María J. Sánchez-Martín, M. Sonia Rodríguez-Cruz

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   Ecotoxicology and Environmental Safety 182 (2019) 109395

6. “Transport of ^{14}C-prosulfocarb through soil columns under different amendment, herbicide incubation and irrigation regimes”

   Víctor Barba, Jesús M. Marín-Benito, María J. Sánchez-Martín, M. Sonia Rodríguez-Cruz

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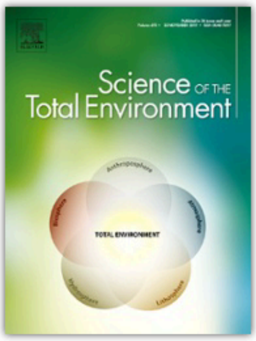

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OBJETIVOS

OBJETIVOS

El objetivo principal del trabajo fue evaluar el efecto de la aplicación de un residuo orgánico (compost vegetal) en suelos de cultivo como enmienda en la disipación, movilidad y persistencia de los herbicidas triasulfuron y prosulfocarb, y la influencia de esta aplicación simultánea en las comunidades microbianas del suelo como indicadores de la conservación y calidad del suelo.

Para conseguir este objetivo se abordaron los siguientes objetivos parciales:

1. Evaluación de la disipación, persistencia y movilidad de los herbicidas triasulfuron y prosulfocarb aplicados en un suelo agrícola sin enmendar y enmendado con compost vegetal en parcelas experimentales en condiciones de campo:

    1.1. Influencia de la aplicación de los herbicidas triasulfuron y prosulfocarb como formulaciones individuales (Logran® y Auros®, respectivamente) o conjunta (Auros Plus®).

    1.2. Evaluación del efecto de la aplicación individual o conjunta de los herbicidas sobre la abundancia, actividad y estructura de las comunidades microbianas del suelo a través de la determinación de los parámetros biomasa microbiana, respiración, actividad deshidrogenasa y perfil de ácidos grasos de fosfolípidos del suelo.

    1.3. Estudio de la aplicación repetida de los herbicidas como una formulación conjunta (Auros Plus®), de la dosis de compost vegetal añadido como enmienda orgánica, del régimen de irrigación y de las propiedades del suelo.

    1.4. Evaluación de la influencia de diferentes factores (dosis de enmienda, riego y aplicación repetida de herbicidas como una formulación conjunta (Auros Plus®)) sobre la estructura de las comunidades microbianas del suelo a través de la determinación del perfil de los ácidos grasos de fosfolípidos.

2. Estudio del mecanismo de disipación y de la movilidad de prosulfocarb en el suelo sin enmendar y enmendado con compost vegetal en condiciones de laboratorio:

    2.1. Evaluación de la disipación del herbicida aplicado a diferentes dosis y balance total de masa del herbicida marcado en ^{14}C. Determinación de las fracciones mineralizada, extraíble en solución acuosa, extraíble en solución orgánica y no extraíble (residuos enlazados) en función del tiempo.

    2.2. Influencia del envejecimiento del herbicida en el suelo y del régimen de lavado de la columna de suelo en la movilidad del ^{14}C-prosulfocarb a través de columnas empaquetadas de suelo sin enmendar y enmendado con compost vegetal.

Los Resultados obtenidos en el desarrollo de estos Objetivos Parciales han dado lugar a seis artículos originales de investigación publicados en revistas científicas de prestigio internacional e indexadas en la edición científica del *Journal Citation Reports* que se incluyen en el ***Apartado 4. RESULTADOS Y DISCUSIÓN*** de esta Memoria en la versión publicada. Un resumen en español de estos artículos se incluye también en dicho ***Apartado 4***.

INTRODUCCIÓN

2. INTRODUCCIÓN

2.1. PESTICIDAS

2.1.1. Producción y uso actual

Un pesticida se puede definir como un compuesto químico, orgánico o inorgánico, cuyo propósito es prevenir, repeler, controlar o mitigar alguna plaga. Una plaga se define, en su sentido más amplio, como cualquier organismo vivo considerado como perjudicial por el hombre y que provoca daños a su persona, a su propiedad o al medioambiente. Puede tratarse de plagas provocadas por animales (ratones, insectos o algún parásito), plantas no deseadas (malas hierbas) o microorganismos (virus, hongos o bacterias).

En la actualidad, la utilización de pesticidas en cultivos agrícolas es esencial para proteger los cultivos frente a cualquier tipo de plaga y producir alimentos de calidad y en suficiente cantidad para cubrir las necesidades alimenticias de la creciente población mundial.

Los pesticidas deben cumplir una serie de requisitos para poder alcanzar un uso amplio en la agricultura: deben ser efectivos contra la plaga sobre la que actúan, no tener efectos negativos sobre otros organismos de la flora o fauna del terreno, su uso debe producir beneficios económicos que superen el gasto que conllevan, no deben ser tóxicos para la salud del hombre ni animales, etc. (Early, 2018). Además, la tendencia actual está dirigida a disminuir las dosis de pesticidas necesarias mediante el desarrollo de formulaciones de compuestos más eficaces que actúan de manera más específica.

Los pesticidas pueden clasificarse según diferentes criterios. La clasificación atendiendo a su aplicación es la más común y engloba a: herbicidas (control de malas hierbas), insecticidas (control de insectos), fungicidas (control de hongos), acaricidas (control de ácaros), bactericidas (control de bacterias), nematicidas (control de nematodos), rodenticidas (control de roedores), etc.

Globalmente, la mayor proporción de pesticidas utilizados en el sector de la agricultura durante el año 2017 se dio en Asia, seguido de América y Europa (FAO, 2017) (Figura 1). España es un país con un alto consumo de pesticidas y con tendencia a crecer con el tiempo. El consumo de pesticidas en nuestro país se incrementó en más de 5500 t en el periodo 2013-2016. Según el Ministerio de Agricultura, Pesca y Alimentación (MAPA, 2017), durante el año 2017 se llegaron a consumir más de 72000 toneladas de pesticidas en agricultura y horticultura con una inversión media de 657 M $ (período

2000-2016). España ocupa el 9º puesto a nivel mundial en lo que a inversión en pesticidas se refiere (FAO, 2017).

Las principales categorías de productos fitosanitarios utilizados en agricultura en nuestro país corresponden al uso de fungicidas y bactericidas (53%), herbicidas (22%), insecticidas y acaricidas (9%) y a otros productos (16%) (Figura 2) (MAPA, 2017).

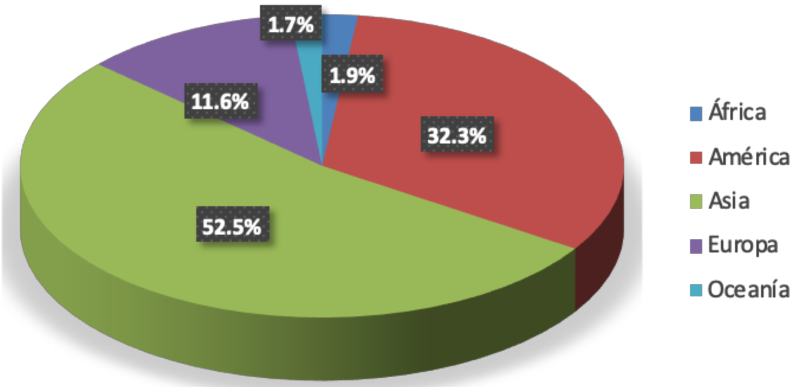

Figura 1. Porcentaje global de ventas de pesticidas en todo el mundo en el año 2017.

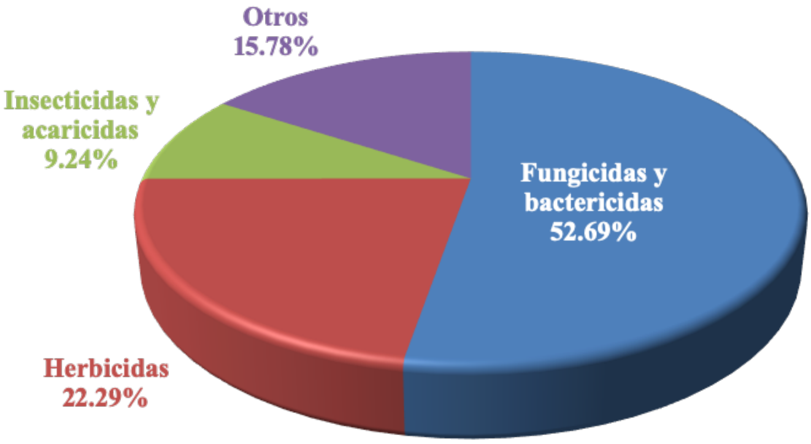

Figura 2. Cantidades (%) de los principales productos fitosanitarios comercializados en España en el año 2017.

2.1.2. Herbicidas

Los herbicidas son compuestos químicos utilizados para inhibir o impedir el desarrollo de plantas no deseadas o malas hierbas en cultivos agrícolas. Estas malas hierbas suelen presentar una alta dispersión (por el viento o el agua) y una gran resistencia. Además, provocan problemas en los cultivos tales como la reducción de la cosecha al competir por recursos como agua o luz, dificultad de la recogida de la cosecha e incremento de los costes económicos.

Los herbicidas pueden clasificarse atendiendo a diferentes criterios:

i) *Según su rango de acción:*

a. Totales: Se aplican para conseguir controlar la totalidad de las malas hierbas presentes. Suelen utilizarse para limpieza de terrenos. Un ejemplo es el glifosato.

b. Selectivos: se aplican para eliminar un tipo concreto de maleza sin afectar al cultivo.

En muchas ocasiones, la clasificación de un herbicida en total o selectivo depende de su concentración o dosis aplicada, dado que un herbicida total puede convertirse en selectivo a concentraciones bajas, y viceversa.

ii) *Según su modo de acción:*

a. Residuales (o de suelo): Se aplican directamente sobre el suelo, creando una película tóxica que regula la proliferación de malas hierbas de tal modo que al atravesarla les provoca la muerte. No suelen afectar a las malas hierbas que ya existían a la hora de su aplicación, sólo a las que germinen.

b. Foliares: Pueden distinguirse dos tipos:

- De contacto: Destruyen las hojas y tallos de las malas hierbas presentes en el terreno donde se aplica al entrar en contacto con ellas y no afectan a las raíces.
- Sistémicos: Son absorbidos y trasladados a través del sistema vascular hasta la raíz, provocando la muerte de las plantas. En este grupo se incluyen los herbicidas hormonales.

iii) *Según el momento de aplicación:*

a. Pre-siembra: Se aplican antes de la siembra.

b. Post-siembra: Se aplican una vez se ha realizado la siembra. Pueden distinguirse dos tipos:

- Pre-emergencia: Se aplican antes de que el cultivo emerja del suelo. Suelen utilizarse en terrenos donde haya historia de presencia de malas hierbas.
- Post-emergencia: Se aplican cuando el cultivo y las malas hierbas ya han germinado.

iv) *Según su composición y estructura químicas:* Compuestos piridínicos y bipiridínicos (paraquat, diquat, etc.), organoclorados (pentaclorofenol, etc.), dinitroanilinas (trifluralin, etc.), sulfonilureas (metsulfurón metilo, triasulfuron, etc.), triazinas (atrazina, etc.) o carbamatos y tiocarbamatos (prosulfocarb, etc.).

A la hora de elegir el herbicida más adecuado se debe tener en cuenta el estado del cultivo y de las malas hierbas a eliminar, además de las características fisicoquímicas del suelo.

Durante el año 2017, en España se utilizaron alrededor de 16000 t de herbicidas en el sector agrícola. Este valor supone un aumento de un 2.5% con respecto al año 2016 y es uno de los más altos registrados en nuestro país donde se ha ido incrementado en la última década (Figura 3) (FAO, 2017).

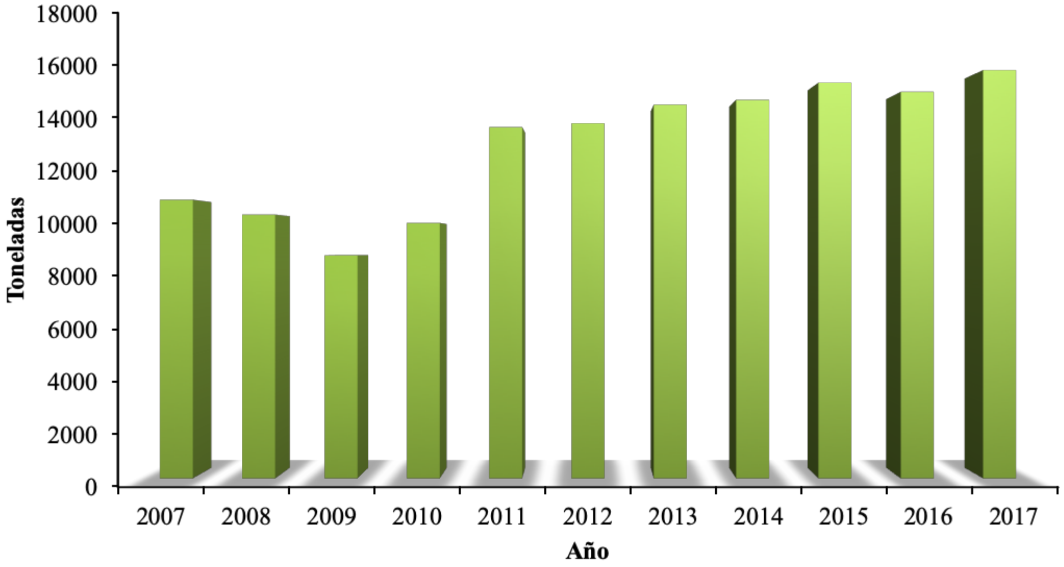

Figura 3. Evolución del uso de herbicidas en España durante los últimos años.

Compuestos pertenecientes al grupo de las sulfonamidas y tiocarbamatos, como el triasulfuron y el prosulfocarb, son ampliamente utilizados en la actualidad en diferentes tipos de cultivos. Estos compuestos pueden utilizarse de manera individual o conjunta para el control de malas hierbas en cereales de secano o regadío, ya que proporcionan un buen control de las malas hierbas (Cirujeda and Taberner, 2010; Knežević et al., 2010; Bajya et al., 2015; Mehmeti et al., 2018).

2.1.3. Contaminación de suelos y aguas por herbicidas

La aplicación de herbicidas en la agricultura actual es una práctica extendida que está dirigida a incrementar el rendimiento de los cultivos. Sin embargo, el uso extensivo de estos compuestos puede plantear serios problemas de contaminación ambiental (Huang et al., 2016; Malyan et al., 2019). Además, el uso de moléculas móviles y persistentes puede afectar a la calidad del suelo y de las aguas superficiales o subterráneas. Por otra parte, la movilidad de los herbicidas desde la superficie del suelo acorta la duración del control residual sobre las malas hierbas y puede producir contaminaciones de las aguas subterráneas como ha sido puesta de manifiesto en sistemas agrícolas de distintos países (Herrero-Hernández et al., 2013; Fingler et al., 2017; Gosetti et al., 2019).

La concentración máxima permitida de pesticidas, incluidos herbicidas, en aguas se encuentra regulada por diferentes directivas que tratan de limitar la contaminación de ecosistemas acuáticos. En la Directiva Europea 80/778/EEC sobre la potabilidad de las aguas y la revisión 98/83/ECC se establece una concentración máxima para un pesticida individual en aguas potables de 0.1 $\mu g\ L^{-1}$, y una concentración total máxima de 0.5 $\mu g\ L^{-1}$. El Reglamento (CE) número 1107/2009 del parlamento europeo, por el que se deroga la directiva 91/414/CEE, establece las normas aplicables a la autorización de productos fitosanitarios en su presentación comercial, y a su comercialización, utilización y control en la Unión Europea. Establece además las normas relativas a la aprobación de sustancias activas, protectores y sinergistas contenidos en los productos fitosanitarios. La finalidad de este reglamento es garantizar la protección de la salud humana y animal, así como el medio ambiente, además de mejorar la producción agrícola.

En la actualidad, sin embargo, se ha observado la presencia de residuos de herbicidas en suelos y aguas en diferentes países del mundo a niveles más altos de los permitidos (Echeverría-Sáenz et al., 2012; Lopez et al., 2015; Zheng et al., 2016; Herrero-Hernández et al., 2017).

Dada la naturaleza tóxica de los herbicidas, se han realizado esfuerzos considerables para controlar, entender y minimizar su impacto medioambiental (Odukkathil and Vasudevan, 2013; Marín-Benito et al., 2018).

2.2. DINÁMICA DE HERBICIDAS EN SUELOS

Los herbicidas aplicados en terrenos agrícolas sufren una dispersión o distribución entre las distintas fases del ambiente: agua, aire, suelo y biota. Estos herbicidas interaccionan con cada una de estas fases de maneras muy complejas a través de multitud de reacciones físicas, químicas o biológicas que suelen ocurrir de manera simultánea. Una vez el herbicida entra en contacto con el suelo empezará a moverse a otros sistemas con su estructura original o degradándose, en función del tiempo.

Los procesos medioambientales que rigen el comportamiento y destino de los herbicidas en suelos pueden dividirse en tres grandes grupos (Zimdahl, 2007; Marín-Benito, 2011):

i) **Acumulación:** Procesos que conllevan el paso entre dos medios sin que el herbicida sufra ninguna transformación química. El herbicida mantiene por lo tanto su función original. En este grupo se incluyen los procesos de adsorción y desorción.

ii) **Eliminación:** Procesos que conllevan una transformación de los herbicidas en otros diferentes que pueden presentar funciones, toxicidades y comportamientos diferentes. Puede tratarse de procesos químicos, biológicos o fotoquímicos. En este grupo se incluye el proceso de degradación.

iii) **Movimiento:** Procesos que conllevan un desplazamiento del herbicida sin incurrir en ninguna transformación química. Se diferencian de los procesos de acumulación en que existe un desplazamiento importante desde la posición inicial. En este grupo se incluyen los procesos de lixiviación, escorrentía y volatilización.

Todos estos procesos pueden ocurrir de manera individual o simultánea y se encuentran determinados no sólo por las propiedades físicas y químicas de los herbicidas sino también por las características del medio con el que interactúa.

El proceso de **adsorción** consiste en el paso de las moléculas de un soluto (denominado adsorbato) desde la fase acuosa hasta la superficie de un sólido que actúa como sorbente (adsorbente) (Calvet et al., 1989). En el caso de los herbicidas, éstos serían el adsorbato y el suelo el adsorbente. Este proceso puede ser de naturaleza física, cuando la adsorción ocurre mediante fuerzas débiles (normalmente fuerzas de van der Waals), o de naturaleza química, cuando ocurre mediante enlace químico. El proceso de adsorción de herbicidas en el suelo está muy relacionado con la superficie específica de las partículas del suelo y su tamaño. Debido a esto, la fracción coloidal del suelo presenta una mayor capacidad de retención de las moléculas del herbicida (Jiang et al., 2018; Spuler et al., 2019).

El proceso de **desorción** consiste en la liberación de moléculas de adsorbato de la superficie del adsorbente. La combinación de estos procesos de adsorción-desorción es muy importante para el comportamiento de los herbicidas en el suelo, ya que condicionan la cantidad de herbicida que se encuentra disponible en el suelo (Silva et al., 2019).

Entre las propiedades fisicoquímicas del suelo que están implicadas en estos procesos de acumulación destacan la textura, el contenido en arcilla y materia orgánica, y el pH del suelo. Por otra parte, las características de los herbicidas también influyen en gran medida en estos procesos, especialmente su volatilidad, solubilidad en agua, persistencia y su estructura química. Además, estos procesos se ven influenciados por diferentes factores del medio, tales como la humedad, temperatura, régimen de lluvias y de vientos, así como por las prácticas de cultivo utilizadas.

El proceso de **degradación** de un herbicida suele provocar la mayor parte de la pérdida de compuesto tras su aplicación (Noshadi et al., 2018; Schuhmann et al., 2019). La degradación puede ser ocasionada por hongos, bacterias y otros microrganismos presentes en el suelo (degradación microbiológica), por diferentes procesos químicos (oxidación, reducción, deshidrohalogenación, hidrólisis) (degradación química) o por la exposición a la luz solar (degradación fotoquímica).

En relación con los procesos que implican un movimiento de los herbicidas en el suelo cabe señalar la **lixiviación** que origina su desplazamiento vertical a través del perfil del suelo. Este proceso es consecuencia de su disolución en agua, ya sea proveniente de la lluvia o del riego y está considerado como el principal responsable de la contaminación de las aguas subterráneas. La lixiviación puede tener lugar mediante un movimiento rápido de los herbicidas en el agua a través de los macroporos del suelo (flujos preferenciales) o mediante un movimiento lento de los herbicidas a través de los microporos del mismo (flujo matricial) (Cohen et al., 1995; Whitford et al., 1995). Otros procesos de movimiento como la **escorrentía** tienen lugar cuando la cantidad de agua aplicada al suelo supera la capacidad de infiltración de dicho suelo. Debido a esto los herbicidas son transportados por la superficie disueltos en el agua o adsorbidos en partículas del suelo pudiendo llegar a alcanzar y/o contaminar las aguas superficiales

(Cohen et al., 1995; Ulrich et al., 2018). Estos compuestos pueden también transportarse por **volatilización** que consiste en la pérdida del herbicida en forma de vapor desde la superficie del suelo, del agua o de las plantas (Voutsas et al., 2005).

2.2.1. Degradación y/o disipación de herbicidas en suelos: Ensayos en laboratorio y en campo

Uno de los principales fenómenos que experimentan los herbicidas en suelo es su degradación a compuestos más sencillos (metabolitos). Por ello, los estudios de degradación, independientemente de la escala a la que se realicen, son esenciales a la hora de evaluar el destino de los herbicidas aplicados en suelos. El escenario más sencillo para el estudio de las cinéticas de degradación es el realizado en condiciones de laboratorio. En la literatura actual puede encontrarse un gran número de estudios de degradación de diferentes herbicidas en suelos con diferentes propiedades en condiciones controladas. Francisco et al. (2018) estudiaron el comportamiento del herbicida aminociclopiracloro en tres suelos con propiedades fisicoquímicas diferentes. Evaluaron la mineralización, los residuos extractables y la formación de residuos enlazados utilizando el herbicida marcado con ^{14}C en condiciones de laboratorio. El uso de compuestos marcados abre nuevas posibilidades de estudio ya que permite, en experimentos realizados en laboratorio, seguir de cerca el destino de esos compuestos al realizar balances de masas y así caracterizar de manera más precisa su comportamiento en los suelos.

En la bibliografía también puede encontrarse trabajos sobre la degradación de estos compuestos aplicados en suelos enmendados con residuos orgánicos. Por ejemplo, Rodríguez-Cruz et al. (2019) estudiaron la degradación del herbicida petoxamida aplicado a un suelo no enmendado y enmendado con lodos de depuradora y compost vegetal en condiciones de laboratorio. Los resultados mostraron que la velocidad de degradación del herbicida disminuía al aumentar la dosis de herbicida aplicado, aunque la vida media del herbicida no presentó diferencias significativas en el suelo sin enmendar y enmendado a las dosis más bajas de herbicida. Haskis et al. (2019) evaluaron la degradación del herbicida metribuzina y sus metabolitos (deamino-, deaminodiketo- y diketo-metribuzina) en condiciones de luz y de oscuridad en muestras de suelo no enmendado y enmendado con biochar. El estudio mostró que la adición del biochar redujo drásticamente o incluso inhibió la degradación durante el periodo de tiempo estudiado.

Los experimentos de degradación realizados en laboratorio permiten que las condiciones del estudio estén controladas. Sin embargo, los resultados son más realistas cuando los estudios se realizan en parcelas agrícolas bajo condiciones de campo, donde los herbicidas, suelos y otros elementos se verán expuestos a condiciones reales del medio. Por ejemplo, Pang et al. (2016) determinaron las cinéticas de disipación de los herbicidas rimsulfuron, mesotriona, fluroxipir-meptil y fluroxipir aplicados a un suelo agrícola cultivado con maíz y determinaron las cinéticas de disipación tanto en muestras de suelo como de paja. Concluyeron que, aunque en un primer momento las cantidades de herbicidas eran mayores en las muestras de paja de maíz que en las de suelo, la velocidad de degradación de los herbicidas era mayor en la paja. Carretta et al (2019) estudiaron la disipación de los herbicidas terbutilazina, su metabolito desetil-terbutilazina, y S-metacloro en condiciones de campo. El experimento se llevó a cabo en parcelas experimentales tras aplicarle un fertilizante inorgánico al suelo, enmendarlo con lodos de depuradora o con una mezcla de ambos. El estudio puso de manifiesto que los herbicidas estudiados presentaban una disipación más rápida en los suelos enmendados solamente con fertilizante inorgánico que en los suelos enmendados con lodos de depuradora o la mezcla de ambos. También se apreció que la presencia de la enmienda orgánica disminuyó la degradación del herbicida terbutilazina y la formación de su metabolito desetil-terbutilazina.

2.2.2. Movilidad de herbicidas en suelos: Ensayos en laboratorio y en campo

Los estudios sobre movilidad de herbicidas en suelos se realizan generalmente en condiciones de laboratorio o invernadero, pudiendo encontrar un gran número de estos trabajos en la literatura actual. Los estudios en laboratorio se basan en el paso de disoluciones de lavado a través columnas de suelo empaquetadas o columnas de suelo sin distorsionar contaminadas con herbicidas y el análisis posterior de las aguas lixiviadas. Berzins et al. (2019) estudiaron la movilidad del herbicida glifosato y su metabolito principal (ácido aminometilfosfónico) en columnas empaquetadas de suelo procedente de la capa superficial de suelos con diferentes características (arenoso y areno-arcilloso). Realizaron tres etapas de lavado de las columnas contaminadas. El contenido total en herbicidas recogido en las tres fases de lavado fue significativamente mayor en las columnas con suelo arenoso que en las columnas con suelo areno-arcilloso. Guimarães et al. (2019) evaluaron la movilidad de los herbicidas ametrin, diuron, hexazinone y metribuzin utilizando columnas empaquetadas con cinco suelos con propiedades fisicoquímicas diferentes. Se aplicaron herbicidas marcados con ^{14}C y determinaron la concentración de dichos herbicidas en las aguas de lavado tras añadir 200 mm durante 48 horas simulando el efecto de la lluvia. Además, determinaron la concentración remanente de los compuestos en las columnas a diferentes profundidades. Concluyeron que la movilidad de los herbicidas dependía especialmente de las características de los suelos estudiados. Vasilakoglou et al. (2000) estudiaron la movilidad de los herbicidas alacloro, acetoclor, metolacloro, S-metolacloro, dimetenamida y flufenacet como distintas formulaciones (emulsionables y microencapsuladas) en columnas empaquetadas de un suelo limo-arcilloso. Según sus resultados, el tipo de formulación afectó a la movilidad de los herbicidas, aunque no detectaron ninguno a profundidades mayores de 30 cm.

Tejada et al. (2017) evaluaron en un suelo semiárido (Xerollic Calciorthid) el efecto de la adición de residuos sólidos municipales, estiércol de aves de corral o estiércol de vaca sobre la movilidad del herbicida flazasulfuron. El estudio se llevó a cabo en columnas empaquetadas con suelo enmendado y sin enmendar y permitió concluir que la aplicación de la enmienda orgánica disminuyó la movilidad del herbicida, especialmente con los residuos sólidos municipales. Otros autores también observaron que la movilidad de otros herbicidas como el linuron se vio afectada tras la enmienda del suelo. Marín-Benito et al. (2013) determinaron que la movilidad del linuron en columnas empaquetadas de un suelo franco arenoso enmendado con diferentes residuos orgánicos (lodos de depuradora, orujo de uva, substrato postcultivo del champiñón) se vio reducida en comparación con el suelo sin enmendar. Por su parte, Marín-Benito et al. (2018) estudiaron el efecto de 5 enmiendas orgánicas, algunas de ellas de bajo coste, utilizadas como barreras en columnas empaquetadas en la movilidad de los herbicidas etofumesato y terbutrina.

Los estudios de movilidad de herbicidas en columnas de suelo sin distorsionar lavadas posteriormente en el laboratorio permiten obtener resultados más representativos de la realidad ya que las propiedades fisicoquímicas (densidad, grado de compactación, tamaño de partícula, micro y macroporosidad, etc.) y microbiológicas del suelo se mantienen inalteradas. A pesar de ello, este tipo de estudios son menos frecuentes que los realizados en columnas empaquetadas. Walker et al. (2005) utilizaron esta técnica para estudiar la movilidad de los herbicidas clortoluron, isoproturon y triasulfuron utilizando columnas sin distorsionar de un suelo franco arenoso y un suelo arenoso franco arcilloso.

Por su parte, López-Piñeiro et al. (2014) estudiaron el efecto de la aplicación de residuos de la molienda de aceitunas como enmienda orgánica en la movilidad de los herbicidas MCPA y S-metalocloro en un suelo franco arcillo-arenoso con bajo contenido en materia orgánica en columnas de suelo inalteradas, observando un movimiento vertical más lento para MCPA y especialmente para S-metacloro en las columnas de suelo enmendado que en las de suelo sin enmendar.

Estos estudios permiten sacar conclusiones rápidas de lo que ocurre en la realidad pero de un modo aproximado. Los resultados más representativos y por tanto más extrapolables requieren estudios de movilidad en campo bajo condiciones de temperatura y humedad reales. Estos estudios se llevan a cabo extrayendo columnas del perfil de suelo donde el herbicida ha sido previamente aplicado y analizando la presencia del compuesto a diferentes profundidades (Boesten and van der Pas, 2000; Mamy et al., 2008; Cabrera et al., 2009) o mediante su recogida en lisímetros instalados en el campo y su cuantificación en los lixiviados (Marín-Benito et al., 2014). El elevado coste económico y tiempo necesario para el desarrollo de este tipo de experimentos junto con su alta dependencia de las condiciones climáticas los hacen menos habituales que los realizados en el laboratorio. Por ejemplo, Kočárek et al. (2010) estudiaron la movilidad del herbicida clortoluron a través del perfil de tres suelos diferentes mediante la toma de muestras sin alterar a diferentes profundidades (0-50 cm). Aunque detectaron la presencia del herbicida a lo largo de todo el perfil estudiado, la mayor concentración del compuesto se encontró en la capa más superficial del suelo (0-2 cm).

2.3. RESIDUOS ORGÁNICOS

2.3.1. Residuos orgánicos y su uso como enmienda en agricultura

Según la legislación española (Ley 10/1998 del 21 de abril), se define *residuo* como “cualquier sustancia u objeto perteneciente a alguna persona de las categorías que figuran en el anexo de esta ley, del cual su poseedor se desprenda o del que tenga la intención u obligación de desprenderse”. Según cual sea el origen de estos residuos, puede hablarse de residuos orgánicos, de origen animal y vegetal (ya sean urbanos, agrícolas, industriales o ganaderos) e inorgánicos (papel, envases, vidrio, metales equipos eléctricos, etc). Los residuos orgánicos suponen alrededor del 80% del total de residuos generados en países desarrollados, por lo que es imprescindible su gestión de manera adecuada para evitar problemas medioambientales y de salud para las personas. Una buena gestión de estos residuos persigue poder aprovechar el valor económico que pueden tener y buscarles una aplicación en lugar de desecharlos.

El aumento de la superficie destinada a la agricultura a nivel mundial, y otros factores como el uso intensivo de pesticidas está acelerando la degradación del suelo y disminuyendo su fertilidad. La disminución en el contenido de materia orgánica que originan modifica a su vez los niveles de nutrientes disponibles en el suelo y su capacidad de retención de agua, debido a una menor porosidad y capacidad de infiltración (FAO, 2017). Por ejemplo, los suelos de Castilla y León presentan un contenido muy bajo en materia orgánica menor del 1% en más del 50% de los suelos (Código de Buenas Prácticas Agrarias de Castilla y León, 1998) y, por tanto, con mayor predisposición hacia fenómenos de degradación y desertización en ellos. Una posible vía de abordar este problema sería la reutilización de residuos orgánicos como enmienda orgánica aplicada en suelos directamente o tras su compostaje. La mejora en las propiedades del suelo debido al contenido en materia orgánica en estos residuos está bien documentada, por lo que su aplicación como enmiendas orgánicas en agricultura es una práctica común (Ferreras et al., 2006; Tejada and González, 2008; Aranda et al., 2015; Bastida et al., 2015) que ha atraído un interés considerable en los últimos años (Scotti et al., 2015).

Una gran variedad de residuos orgánicos pueden ser aplicados como enmiendas orgánicas en la agricultura entre los que cabe destacar los residuos urbanos (lodos de depuradora), los derivados de la industria agroalimentaria (los residuos generados en el cultivo del champiñón y setas comestibles), o de la producción agrícola y ganadera (restos de cosechas, podas y purines). Los residuos generados en las podas de parques y jardines, o compost vegetal (Figura 4) han experimentado un gran crecimiento en los últimos años en España. En 2015, 229300 toneladas of compost vegetal se generaron en España, lo cual representa un 1.13% de los residuos urbanos (MAPA, 2017). Estos residuos vegetales son biodegradables, potencialmente aprovechables y presentan un contenido en materia orgánica superior al 15% en peso seco tras someterse a un proceso de compostaje. Por ello, pueden considerarse como una fuente de materia orgánica para el suelo más estable que la proporcionada por otros residuos biodegradables, como los biosólidos o los lodos de depuradora.

Figura 4. a) Compost vegetal; b) Parcelas agrícolas enmendadas con compost vegetal.

El uso de este compost vegetal presenta ciertas ventajas al aplicarse como enmienda orgánica: es una alternativa económica y relativamente sencilla logísticamente para dar salida a estos residuos procedentes de las podas de plantas urbanas, disminuye potencialmente la producción de metano por parte de los residuos orgánicos al llevarse a cabo un compostaje aerobio, mejora la fertilidad del suelo al aumentar el contenido de materia orgánica, y disminuye la necesidad de fertilizantes inorgánicos e irrigación. En España, su utilización como fertilizante o enmienda orgánica del suelo se encuentra regulada por el Real Decreto 999/2017, de 24 de noviembre, por el que se modifica el Real Decreto 506/2013, de 28 de junio, sobre productos fertilizantes (BOE 296, 6 diciembre 2017).

2.3.2. Efecto de residuos orgánicos en el comportamiento de herbicidas

La aplicación de residuos orgánicos al suelo implica la inclusión de nueva materia orgánica en el mismo para mejorar su calidad. Sin embargo, esta nueva materia orgánica exógena (sólida y líquida) de distinta naturaleza a la presente en el suelo puede influir en el comportamiento de los herbicidas aplicados (Marín-Benito et al., 2012, 2013, 2014). Estos herbicidas pueden ser adsorbidos por los suelos enmendados ya que el aumento de materia orgánica sólida facilitará un aumento de adsorción de los pesticidas no-iónicos, hidrofóbicos, con solubilidad baja o muy baja en agua (Huang et al., 1995), lo que puede tener consecuencias para su degradación, persistencia y movilidad (Rodríguez-Liébana et al., 2014; Marín-Benito et al., 2016; Keren et al., 2017). Estos procesos pueden disminuir la contaminación por parte de estos herbicidas tanto en el suelo como en las aguas subterráneas, pero también pueden afectar a la concentración final que se encuentra biodisponible para su absorción por parte de las malas hierbas a las que van destinados (Barriuso et al., 1997). Sin embargo, el aumento de materia orgánica líquida podría aumentar la movilidad de estos compuestos, mediante el establecimiento de interacciones hidrofóbicas con la materia orgánica disuelta en el agua facilitando su solubilización y transporte en el suelo, con la consiguiente contaminación de las aguas subterráneas (Kozak, 1996; Cox et al., 2007).

En la actualidad se realizan muchos estudios sobre la influencia de los residuos orgánicos de diferente origen (estiércol, lodos procedentes de aguas residuales, residuos agroindustriales procedentes de la elaboración de vino, aceite o el cultivo del champiñón) en la adsorción, movilidad y degradación de pesticidas en los suelos enmendados (Gámiz et al., 2012; Cheng et al., 2017; James es al., 2019). Algunos abordan también el estudio de la influencia de la materia orgánica disuelta procedente de estos residuos en el comportamiento de los pesticidas en el suelo (Cox et al., 2001; Barriuso et al., 2011) o la influencia en la biodisponibilidad de los residuos en los suelos enmendados (Gevao et al., 2001; Barriuso et al., 2004).

Se han llevado a cabo algunos estudios para evaluar el efecto del compost vegetal en la adsorción y disipación de los herbicidas mesotriona, triasulfuron, petoxamida, flufenacet y clortoluron en suelos agrícolas enmendados con este residuo en condiciones de laboratorio (Marín-Benito et al., 2019; Pose-Juan et al., 2015, 2017, 2018; Rodríguez-Cruz et al., 2019). En estos trabajos se indicó que la disipación de los herbicidas en suelos enmendados con compost vegetal estaba controlada principalmente por el proceso de adsorción que aumenta con la aplicación de la enmienda orgánica al suelo, disminuyendo la biodisponibilidad de los compuestos y la velocidad de degradación de los herbicidas.

2.3.3. Efecto de residuos orgánicos sobre las comunidades microbianas del suelo

La aplicación de residuos orgánicos como enmiendas, con el objetivo de mejorar la fertilidad y estabilidad del suelo, provoca además la estimulación del crecimiento y biomasa microbiana (Bastida et al., 2015). El uso de enmiendas orgánicas puede incluir además nuevos microrganismos al suelo y promover el crecimiento de microrganismos específicos que modifiquen la estructura, actividad, funcionamiento y diversidad microbianas (García-Delgado et al., 2015; Álvarez-Martín et al., 2016; Sun et al., 2017). El tipo de enmienda aplicada determinará este incremento en la biomasa microbiana.

Después de la aplicación de diferentes residuos orgánicos en suelos se ha observado un efecto estimulatorio en la actividad microbiana determinada por un incremento de la actividad deshidrogenasa (Marín-Benito et al., 2014; Álvarez-Martín et al., 2016; Pose-Juan et al., 2018). Sin embargo, se han observado casos en los que la actividad deshidrogenasa disminuía o aumentaba en el tiempo en presencia de herbicidas y enmiendas orgánicas, según su disipación y / o metabolismo en el suelo enmendado (Pose-Juan et al., 2017).

2.3.4. Efecto de los herbicidas sobre las comunidades microbianas del suelo

La actividad microbiana es un indicador preciso de la calidad del suelo, ya que los microrganismos presentes juegan un papel clave en la descomposición de la materia orgánica y en los ciclos biogeoquímicos que afectan a la fertilidad del suelo (Pascual et al., 2000; García-Orenes et al., 2013; Swarcewicz et al., 2013). En la agricultura intensiva moderna, debido a la aplicación de grandes cantidades de herbicidas durante el periodo de crecimiento del cultivo (Nyamwasa et al., 2018), se han detectado residuos de estos compuestos en suelos de áreas agrícolas en un amplio rango de concentraciones (Li et al., 2014; Pose-Juan et al., 2015), lo que puede provocar cambios en la biodiversidad microbiana del suelo. Estos residuos dependen de su disipación en el suelo, viéndose modificados por diferentes factores ambientales (tipo de suelo, materia orgánica del suelo, régimen de temperatura y precipitaciones, irrigación, etc.), por la formulación de los pesticidas y el método de aplicación (una dosis o dosis repetidas) (Arias-Estévez et al., 2008).

El uso extensivo de los herbicidas puede llevar a una disminución en la biodiversidad microbiana del suelo, con un impacto negativo en el rendimiento de los cultivos (Baxter and Cummings, 2008), lo que puede agravarse por la pérdida de materia orgánica del suelo que se ha detectado en los últimos años (Pascual et al., 2000). Las comunidades microbianas del suelo son sensibles a algunos herbicidas, como las sulfonilureas, que afectan a sus procesos biológicos (Patyka et al., 2016; Wang et al., 2018). Las concentraciones retenidas en el perfil del suelo pueden superar el umbral conocido para las sensibilidades de las especies susceptibles.

Hoy en día, obtener información sobre los efectos que tienen los herbicidas sobre los microrganismos del suelo y la evaluación de la toxicidad de estos compuestos para las comunidades microbianas es un requisito indispensable para mejorar la regulación de la utilización de los herbicidas a corto plazo (Karpouzas et al., 2014). Según la literatura, la presencia de herbicidas y su degradación en suelos puede inhibir, promover, o ser inocua a la diversidad microbiana y sus funciones (Hussain et al., 2009; Imfeld and Vuilleumier, 2012). Por lo tanto, considerando la importancia de la biomasa microbiana, su diversidad y actividad en muchos ciclos del suelo, y por tanto, en su salud, existe un importante interés científico en determinar el impacto que los herbicidas tienen en las comunidades microbianas del suelo (Martin-Laurent et al., 2010).

Algunos trabajos inciden en la capacidad que presentan los microorganismos del suelo para degradar los herbicidas presentes en el suelo (Hussain et al., 2007) utilizando estos compuestos como fuente de energía y nutrientes para incrementar el tamaño de la población microbiana del suelo (Tyess et al., 2006). Más recientemente, estudios sobre los cambios en la composición microbiana y/o estructura y funcionamiento de la comunidad mediante la utilización de técnicas moleculares de extracción de ADN del suelo, amplificación (PCR) y diferenciación por electroforesis en gel con gradiente desnaturalizante (DGGE) (Wang et al., 2009; Sopeña and Bending, 2013), perfiles de ácidos grasos de fosfolípidos (Cycoń et al., 2013; Pose-Juan et al., 2017), o técnicas similares (Widenfalk et al., 2008) han suscitado un gran interés.

2.3.5. Efecto de la aplicación de residuos orgánicos y herbicidas de manera simultánea sobre las comunidades microbianas del suelo

No existe mucha información en la literatura sobre la respuesta y funcionamiento de las comunidades microbianas del suelo al aplicar de manera simultánea pesticidas y residuos orgánicos. La mayoría de los estudios publicados al respecto han sido realizados bajo condiciones de laboratorio o invernadero (Cycoń et al., 2013; Karpouzas et al., 2014), mientras que de nuevo los experimentos en condiciones de campo son muy escasos (Spyrou et al., 2009; Petric et al., 2016).

Dado que la aplicación de enmiendas orgánicas afecta al comportamiento de los herbicidas en el suelo, podrán regular su biodisponibilidad a los microrganismos y su concentración, afectando a su posible impacto sobre las comunidades microbianas.

En la última década el creciente interés por conocer estos efectos ha propiciado un aumento de las publicaciones en la bibliografía mundial, tal como se indica en algunas revisiones recientes sobre el tema (Bünemann et al., 2006, Gianfreda y Rao, 2008, 2011; Hussain et al., 2009). Generalmente se determinan parámetros de las comunidades microbianas indicadores de la abundancia, como la biomasa microbiana del suelo (Dutta et al., 2010), o de su actividad o funcionamiento, como la respiración del suelo (Joseph et al., 2010; Cycoń et al., 2012) o las actividades enzimáticas, siendo las más estudiadas las oxidorreductasas, como la deshidrogenasa, y las hidrolasas, como ureasa y fosfatasa (Romero et al., 2010). Esta aplicación combinada de enmiendas orgánicas y pesticidas puede incrementar o reducir la abundancia relativa de bacterias y hongos durante el tiempo de disipación de los herbicidas en suelos enmendados según el tipo de enmienda y el tiempo de incubación (Pose-Juan et al., 2017).

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MATERIALES Y MÉTODOS

3. MATERIALES Y MÉTODOS

3.1. MATERIALES

3.1.1. Compost vegetal

Se han utilizado dos tipos de compost vegetal (GC) como enmiendas orgánicas en los estudios realizados. Ambos proceden del compostaje de residuos de origen vegetal procedentes de la poda de plantas y árboles en parques y jardines. El primero (GC1) fue suministrado por el Ayuntamiento de Salamanca y el segundo (GC2) por los viveros “El Arca S.L.” en Salamanca (Figura 5). Sus principales características fisicoquímicas referidas a materia seca se recogen en la Tabla 1.

Figura 5. Compost vegetales (GC1 y GC2).

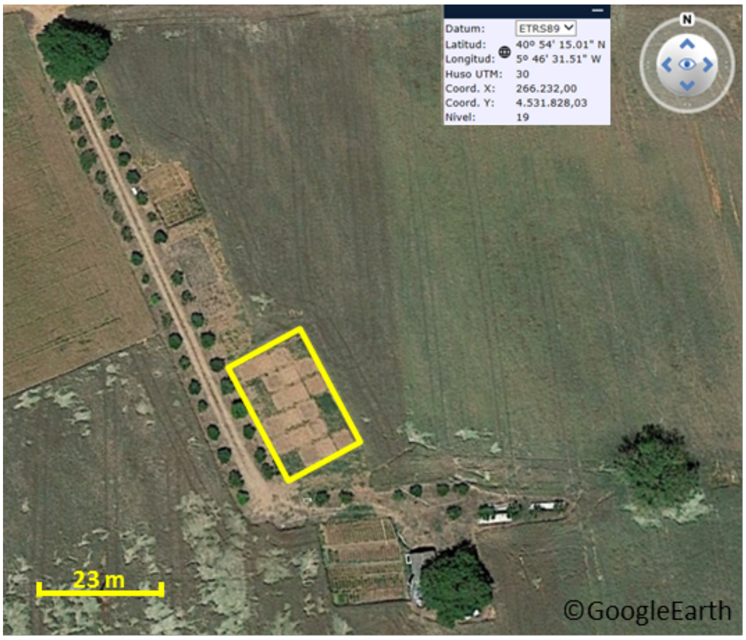
Tabla 1. Características de los compost vegetales

Propiedades	GC1	GC2
pH	7.33	7.58
[a]CO (%)	9.80	24.1
[b]COD (%)	0.35	0.70
N (%)	1.04	1.10
C/N	9.42	21.9
Cenizas (%)	74.5	54.0

[a] carbono orgánico; [b] carbono orgánico disuelto

3.1.2. Suelo

Los experimentos de campo y laboratorio se llevaron a cabo en un suelo franco arcillo-arenoso (58% arena, 17% limo, 25% arcilla, 0.21% $CaCO_3$) (Typic Haploxerept) (Soil Survey Staff, 2010) situado en la finca experimental Muñovela perteneciente al Instituto de Recursos Naturales y Agrobiología de Salamanca (IRNASA-CSIC). Se diseñaron parcelas experimentales en este suelo sin enmendar (S) y enmendado con los dos compost vegetales indicados en el apartado anterior (S+GC1; S+GC2) (Figura 6). Las principales características de este suelo sin enmendar y enmendado se incluyen en la Tabla 2.



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Longitud: 5º 46' 31.51" W
Huso UTM: 30
Coord. X: 266.232,00
Coord. Y: 4.531.828,03
Nivel: 19

Figura 6. Fotografía aérea de las parcelas experimentales diseñadas en la finca experimental Muñovela (Barbadillo, Salamanca).

Tabla 2. Características del horizonte superficial del suelo (0-10 cm) sin enmendar y enmendado.

Suelo	pH	CO (%)	COD (%)	N (%)	C/N	Mineralogía[a]
S	7.35	1.30	0.006	0.12	10.8	K, I, M
S+GC1	7.77	1.98	0.007	0.19	10.6	
S+GC2	7.30	4.66	0.027	0.42	11.0	

[a] K, Caolinita; I, Ilita; M, Montmorillonita.

3.1.3. Herbicidas

Los diferentes estudios realizados se han llevado a cabo con dos herbicidas pertenecientes a dos grupos químicos distintos: triasulfuron y prosulfocarb. Estos herbicidas presentan diferentes características químicas y se aplican habitualmente en cultivos de cereal de Castilla y León.

Triasulfuron

Triasulfuron (2-(2-cloroetoxi)-N-(((4-metoxi-6-metil-1,3,5-triazin-2-il) amino) carbonil) bencenosulfonamida) es una sulfonilurea con actividad herbicida selectiva para el control en pre- y post-emergencia de especies de hoja ancha en cultivos de cebada, avena y trigo.

El triasulfuron es un ácido débil (pKa 4.8) que se presenta de manera predominante en forma de anión a pH alto. Su peso molecular es 401.8 g mol^{-1}. Tiene una elevada solubilidad en agua (815 mg L^{-1} a 20ºC) y baja hidrofobicidad por lo que presenta una alta movilidad en suelos. Este herbicida actúa inhibiendo la actividad de la enzima acetolactato sintasa, responsable de la biosíntesis de aminoácidos de cadena ramificada (leucina, isoleucina y valina) en plantas y bacterias. Su estructura química y algunas de sus características se incluyen en la Tabla 3.

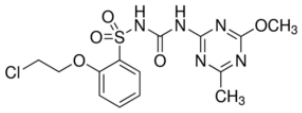
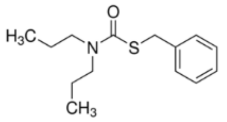
Prosulfocarb

Prosulfocarb (S- (fenilmetil) dipropilcarbamotioato) es un tiocarbamato sistémico con actividad selectiva al aplicarse de manera temprana en pre- y post-emergencia contra hierbas y malezas de hoja ancha en cultivos como la cebada y el trigo de ciclo largo.

El prosulfocarb es un herbicida hidrófobico con alta adsorción, baja movilidad y moderada persistencia en suelo. Su peso molecular es 251.39 g mol^{-1}. Este herbicida actúa inhibiendo la enzima elongasa. Por ello su principal efecto es la inhibición de las síntesis de ácidos grasos de cadena muy larga (lípidos), aunque también afectan a los tejidos meristemáticos. Además, actúa inhibiendo la síntesis del ácido shikímico como efecto secundario, lo que conlleva una disminución del contenido de flavonoides y una variación de la composición y contenido de aminoácidos. Su estructura química y algunas de sus características se incluyen en la Tabla 3.

El estándar analítico de ambos compuestos fue de PESTANAL® (pureza > 98.9%) y suministrado por Sigma Aldrich Química S.A. (Madrid, España). El prosulfocarb se utilizó además marcado en ^{14}C (actividad específica 3.16 MBq mg^{-1}, pureza 94.8%) y fue suministrado por IZOTOP Co. Ltd (Budapest, Hungría). Para el estudio de campo se utilizaron las formulaciones comerciales individuales de triasulfuron (Logran® 20% p/p) y prosulfocarb (Auros® 80% p/v) y además se utilizó la formulación combinada de ambos herbicidas (Auros Plus®). Estos productos comerciales fueron suministrados por Syngenta Agro S.A (Madrid, España).

Tabla 3. Características de los herbicidas (PPDB, 2019).

	Triasulfuron	Prosulfocarb
Nombre (IUPAC)	1-[2-(2-chloroethoxy) phenylsulfonyl]-3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl) urea	*S*-benzyl dipropyl(thiocarbamate)
Estructura química		
Solubilidad agua (mg L^{-1})	815 (20ºC, pH 7)	13.0 (20ºC, pH 6.1)
log K_{ow}	-0.59 (20ºC, pH 7)	4.48 (20ºC, pH 7)
DT_{50} (laboratorio) (días)	59.1	12.4
DT_{50} (campo) (días)	38.5	9.8
pKa	4.64 (25ºC)	-
Índice GUS	5.12	0.84

3.2. MÉTODOS EXPERIMENTALES

3.2.1. Caracterización del compost vegetal y del suelo sin enmendar y enmendado

Las características del compost vegetal se determinaron en muestras molidas y tamizadas en un tamiz de malla con luz < 2 mm. Su pH se midió en una suspensión del compost en agua destilada en una proporción 1:2.5 (peso:volumen). Su humedad se determinó mediante diferencia de peso tras secar 5 g de los residuos a una temperatura de 110 ºC durante 24 h. El contenido en cenizas se determinó por diferencia de peso después de calcinar 5 g de los residuos a una temperatura de 540ºC. El carbono orgánico disuelto (COD) se determinó en una suspensión del residuo en agua ultrapura Milli-Q con una relación 1:100 (peso:volumen) tras someterse a una agitación durante 24 h a 20ºC. La suspensión se centrifugó durante 20 min a 10000 rpm y posteriormente se filtró a través de filtros de nylon de 0.45 μm (Sartorius Stedim Biotech, Alemania). La determinación se realizó en un analizador de carbono Shimadzu TOC-VCSH (Shimadzu, Columbia, MD, Estados Unidos) a partir de rectas de calibrado de disoluciones patrón preparadas con ftalato potásico de diferentes concentraciones (entre 1 y 1000 μg mL^{-1}). El contenido en carbono orgánico total (CO) y de nitrógeno (N) se determinó en un analizador elemental LECO CN628 (LECO Corporation, Saint Joseph, USA).

Las características de los suelos se determinaron siguiendo los métodos habituales de análisis de suelos en muestras tamizadas utilizando un tamiz de malla de 2 mm (Sparks, 1996). La composición granulométrica de los suelos se determinó mediante el método de la pipeta de Robinson. El CO y N se determinaron con el analizador elemental LECO CN628. El COD se determinó mediante el mismo procedimiento indicado para el compost vegetal pero utilizando una relación 1:2 (peso:volumen). El carbono inorgánico, expresado como $CaCO_3$, se determinó mediante el Calcímetro de Bernard. Los minerales de arcilla se identificaron cualitativamente por difracción de rayos X usando un difractómetro Philips PW 1710 (Eindhoven, Países Bajos) (Robert, 1975).

3.2.2. Estudio de disipación y movilidad de los herbicidas triasulfuron y prosulfocarb en el suelo sin enmendar y enmendado: Experimentos de campo

Se estudió la disipación y movilidad de los herbicidas triasulfuron y prosulfocarb aplicados en las parcelas experimentales como formulaciones comerciales de los herbicidas en forma individual y conjunta. Se realizaron dos experimentos para estudiar la influencia de diferentes factores en los procesos indicados. En ambos experimentos se analizó la variación de la concentración de los herbicidas en función del tiempo, bien en la capa superficial del suelo (0-10 cm) para estudiar su cinética de disipación o a lo largo del perfil del suelo (0-50 cm) para estudiar su movilidad. En ambos procesos se estudió:

i) La influencia de la aplicación del GC como enmienda del suelo y de la aplicación individual o conjunta de los herbicidas

Para este estudio se establecieron 24 parcelas experimentales de 9 m^2 de superficie (3m × 3m) de manera aleatoria con tres réplicas por tratamiento. De dichas parcelas, 12 estaban compuestas por suelo sin enmendar y 12 estaban compuestas por suelo enmendado con GC1 con una dosis de 120 t ha^{-1} en peso seco (~ 11.6 t C ha^{-1}). Previamente a la enmienda, el suelo fue labrado y el GC incorporado en la capa superficial del suelo (0-20 cm) de cada parcela. Tres parcelas sin enmendar y enmendadas fueron tratadas con las formulaciones comerciales individuales de cada uno de los herbicidas (Logran® y Auros®) y con la formulación comercial combinada de ambos (Auros Plus®). Los herbicidas fueron aplicados como soluciones acuosas (5 L) de forma manual usando una mochila acoplada a un pulverizador (8 salidas) poco después de la enmienda. Las dosis aplicadas, tanto en las formulaciones individuales como en la conjunta, fueron de 100 g i.a. ha^{-1} para el triasulfuron y de 4.5 kg i.a. ha^{-1} para el prosulfocarb (dosis recomendadas). Otras tres parcelas, tanto sin enmendar como enmendadas, fueron utilizadas como controles y no fueron tratadas con los herbicidas.

ii) La influencia de la dosis de GC aplicado como enmienda del suelo, riego y dosis repetidas de herbicidas

El diseño experimental para este estudio consistió en 6 parcelas de suelo sin enmendar y 12 parcelas de suelo enmendado con GC. Se utilizaron dos GC (GC1 y GC2) para conseguir diferente contenido de materia orgánica en las parcelas. Seis de las parcelas fueron enmendadas con una dosis de 120 t ha^{-1} en peso seco (~ 12 t C ha^{-1}, S+GC1) y otras 6 parcelas con una dosis de 180 t ha^{-1} en peso seco (~ 40 t C ha^{-1}, S+GC2). Las parcelas experimentales se dispusieron de manera aleatoria. Los residuos orgánicos fueron incorporados a la capa superficial del suelo (0-20 cm). En cada tratamiento, tres parcelas recibieron riego adicional de 2.8 mm por semana (123.2 mm en total), mientras que las otras sólo recibieron agua por medio de la lluvia. Las parcelas fueron tratadas con la formulación comercial conjunta de ambos herbicidas (Auros Plus®) de manera similar al estudio anterior. Las dosis aplicadas fueron de 250 g i.a. ha^{-1} para el triasulfuron y 11.25 kg i.a. ha^{-1} para el prosulfocarb (2.5 veces la dosis agronómica de los mismos). Se realizó una segunda aplicación de los herbicidas en las parcelas cuando la disipación de los herbicidas superó el 50% de la cantidad inicialmente aplicada (68 días).

3.2.3. Toma de muestras de suelos

Para estudiar la disipación de los herbicidas se tomaron muestras de suelo del tramo superficial (0-10 cm) de las parcelas experimentales a diferentes tiempos después de su aplicación. El primer muestreo tuvo lugar 24 h después de la aplicación de los herbicidas para determinar la concentración inicialmente aplicada. Se tomaron 5 submuestras de cada parcela que fueron mezcladas antes de ser transportadas en contenedores de polipropileno (Figura 7a).

Para estudiar la movilidad de los herbicidas se tomaron columnas de suelo hasta 50 cm de profundidad (5 en cada parcela). Cada columna fue dividida en 5 tramos de 10 cm cada uno y se prepararon muestras medias mezclando la muestra correspondiente a cada tramo de las 5 columnas de cada parcela. Las muestras mezcladas correspondientes a cada uno de los tramos se transportaron en contenedores de polipropileno (Figura 7b).

Figura 7. Toma de muestra superficial (a) y en profundidad (b) en las parcelas experimentales.

3.2.4. Extracción y análisis cuantitativo de los herbicidas

La extracción de los herbicidas se llevó a cabo en el laboratorio a partir de 6 g de muestra de suelo, por duplicado, tratadas con 12 mL de metanol. La mezcla se sometió a un proceso de agitación (sonicación) durante 1 h y posteriormente se mantuvo en agitación mecánica intermitente durante 24 h en cámara termostatizada a 20ºC.

Posteriormente se centrifugó a 3000 rpm durante 15 min y se filtró mediante filtros de nylon de 0.45 µm. Se tomó un volumen de 8 mL y se evaporó hasta sequedad bajo flujo de nitrógeno en un evaporador EVA-EC2-L (VLM GmbH, Bielefeld, Alemania). Los residuos se disolvieron en 0.75 mL de metanol con ácido fórmico (1%) y se transfirieron a viales de HPLC para su posterior análisis. La determinación cuantitativa de los herbicidas se realizó mediante cromatografía líquida de alta eficacia acoplada a un detector de masas (HPLC-MS).

El análisis de los herbicidas se llevó a cabo en un cromatógrafo Waters (Waters Assoc., Milford, MA, Estados Unidos) equipado con un sistema de bombeo multisolvente modelo e2695 y un inyector automático acoplado a un espectrómetro de masas Micromass ZQ (MS) equipado con una interfaz de electrospray (ESI) y un sistema para la adquisición y procesamiento de datos Empower Pro (Waters Technologies) (Figura 8). Se utilizó una columna Luna® 3 µm PFP(2) 100 Å (150 × 4.6 mm) (Phenomenex, Torrance, CA, Estados Unidos) a temperatura ambiente.

Figura 8. Equipo de cromatografía líquida HPLC-MS.

Se utilizó una fase móvil compuesta por un 70% de acetonitrilo de grado HPLC y un 30% de agua ultrapura Milli-Q (millipore) con 1% de ácido fórmico. La velocidad de flujo de la fase móvil fue 0.4 mL min^{-1} y el volumen de inyección fue 10 µL. Se utilizó una temperatura de 120 ºC para la fuente y se fijó la temperatura de desolvatación en 300ºC. Se aplicó un voltaje de cono de 20V, un voltaje del capilar de 3.08 kV, un voltaje del extractor de 5 V y un voltaje de las lentes de 0.3 V.

Para la detección del triasulfuron, se buscó la relación m/z de 402.8 correspondiente al ion molecular positivo $[M+H]^+$. Su tiempo de retención bajo las condiciones anteriormente descritas fue de 6.1 min y sus límites de detección (LOD) y cuantificación (LOQ) fueron 0.018 $\mu g\ mL^{-1}$ y 0.059 $\mu g\ mL^{-1}$, respectivamente. En el caso del prosulfocarb, su ion molecular positivo presentó una relación m/z de 252.4 y su tiempo de retención fue 14.1 min. Su LOD y LOQ fueron 0.005 $\mu g\ mL^{-1}$ y 0.017 $\mu g\ mL^{-1}$, respectivamente.

El análisis cuantitativo de las cantidades de los herbicidas presentes en las disoluciones se llevó a cabo a partir del área bajo el pico de cada compuesto en modo SIM. El equipo determinó automáticamente las rectas de calibrado considerando las áreas de los picos eluidos correspondientes a los patrones de diferentes concentraciones. Las rectas de calibrado utilizadas se prepararon en soluciones de extractos de los adsorbentes para evitar un posible efecto matriz.

3.2.5. Determinación de parámetros indicadores de la estructura, abundancia y actividad de las comunidades microbianas durante el proceso de disipación de los herbicidas

Análisis del perfil de ácidos grasos de fosfolípidos del suelo

La composición o estructura de las comunidades microbianas del suelo de las parcelas experimentales se llevó a cabo a partir del análisis del perfil de ácidos grasos de fosfolípidos (Frostegård et al., 1993) en las muestras superficiales (0-10 cm) de suelo sin enmendar y enmendado, y tratadas o sin tratar con los herbicidas. Las muestras de suelo fueron liofilizadas inmediatamente después de ser tomadas. Posteriormente, se pesaron 2 g de material seco por duplicado y se utilizaron para la extracción de los lípidos. Los lípidos fueron extraídos con una disolución tampón de cloroformo-metanol-fosfato. Los fosfolípidos se separaron de otros lípidos no polares y se transformaron en ésteres metílicos de ácidos grasos antes de su análisis. La cuantificación de los fosfolípidos se llevó a cabo usando un cromatógrafo de gases modelo Agilent 7890 (Agilent technologies, Wilmington, DE, Estados Unidos) equipado con una columna de 25 m Ultra 2 (5% fenil)-metilpolisiloxano (J&W Scientific, Folsom, CA, Estados Unidos) y un detector de ionización de llama. Los fosfolípidos fueron identificados utilizando estándares de ácidos grasos bacterianos y un software de sistema de identificación microbiano (Microbial ID, Inc., Newark, DE, Estados Unidos). Se utilizaron algunos ácidos grasos específicos como biomarcadores (Zelles, 1999) para cuantificar la abundancia relativa de bacterias Gram negativas (ácidos grasos monosaturados y ciclopropil 17:0) y Gram positivas (ácidos grasos ramificados saturados iso y anteiso), Actinobacterias (ácidos grasos 10-metil) y hongos (18:2 ω6 cis).

Biomasa microbiana del suelo

La biomasa del suelo se determinó a partir del contenido en nitrógeno presente en la biomasa. El nitrógeno de la biomasa fue extraído del suelo mediante la técnica de fumigación-extracción con cloroformo durante 7 días (Vance et al., 1987). El contenido del citoplasma fue extraído con una disolución de K_2SO_4 (Figura 9a) y el amonio presente fue analizado por colorimetría con un autoanalizador de flujo segmentado modelo AutoAnalyzer 3 (Bran+Luebbe GmbH, Norderstedt, Alemania). Los resultados se expresaron como mg C kg^{-1} de suelo seco, convirtiendo las concentraciones de nitrógeno a carbono con un factor de 21 (García-Izquierdo, 2003).

Respiración del suelo

La respiración del suelo es un indicador del nivel de estrés que una perturbación puede provocar en los microorganismos del suelo. Este parámetro se determinó a partir de la depresión originada por el consumo de oxígeno por parte de los microorganismos. La absorción de oxígeno por parte de los microorganismos del suelo se cuantificó en muestras de 50 g de suelo durante un periodo de 4 días utilizando un equipo OxiTop Control OC 110 (WTW, Weilheim, Alemania) (Figura 9b). El CO_2 producido por el metabolismo de los microorganismos del suelo fue retenido en una disolución de 10 mL de NaOH 1M. Aplicando la ecuación de los gases ideales se obtienen los moles de oxígeno y los resultados se expresan como mg O_2 g^{-1} de suelo seco.

Actividad deshidrogenasa del suelo

Se determinó la actividad deshidrogenasa del suelo como un indicador de la actividad microbiana total. Se llevó a cabo siguiendo el método de Tabatabai (1994) a los distintos tiempos de muestreo. Se pesaron 6 g de muestra de suelo por duplicado y se mezclaron con 0.06 g de $CaCO_3$, 1 mL de una disolución acuosa de cloruro de 2,3,5-trifeniltretrazolio (TTC) al 3% y 2.5 mL de agua ultrapura Milli-Q (Millipore). La reacción se incubó a 37ºC en oscuridad y condiciones anaerobias durante 24 h. Tras este tiempo se extrajo el compuesto 1,3,5-trifenilformazan (TPF) originado en el proceso mediante tres extracciones seguidas con 7 mL de metanol. Las tres fracciones fueron mezcladas y diluidas hasta 25 mL con metanol. Los reactivos TTC y TPF fueron suministrados por Sigma-Aldrich Química S.A. (Madrid, España). La actividad deshidrogenasa se determinó a partir de la medida de la absorbancia del compuesto TPF a la longitud de onda de 485 nm en un espectrofotómetro UV-visible modelo Cary 100 Conc (Varian Optical Spectroscopy Instruments) (Figura 9c). Los valores se expresan en μg TPF g^{-1}de suelo seco.

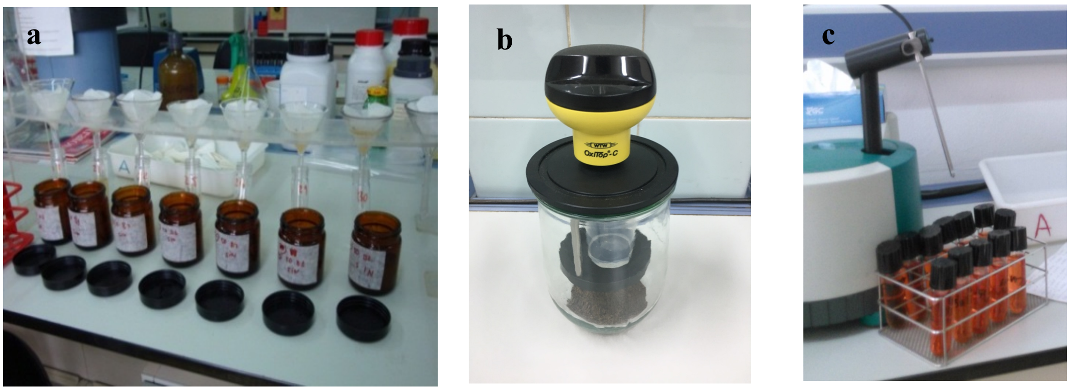


Figura 9. Determinación de: a) biomasa microbiana; b) respiración; y c) actividad deshidrogenasa del suelo.

3.2.6. Estudios de la disipación y movilidad de prosulfocarb en el suelo sin enmendar y enmendado: Experimentos de laboratorio

Disipación del herbicida

Se estudió la disipación del herbicida prosulfocarb en muestras de suelo sin enmendar y enmendado con compost vegetal GC2 en condiciones de laboratorio utilizando prosulfocarb no marcado y marcado en ^{14}C. El suelo sin enmendar procedía del horizonte superficial (0-30 cm) de la parcela experimental de la Finca Muñovela (parcelas control sin enmienda y sin tratar con los herbicidas). El suelo enmendado se preparó incorporando el compost vegetal GC2 de manera uniforme en una proporción de 20% p/p (~180 t ha^{-1}). El suelo y el GC se tamizaron (< 2 mm) previamente a su mezcla. Los suelos enmendados se incubaron durante 10 días a una temperatura de 20ºC en cámara termostatizada para estabilizar el suelo enmendado. Se determinó la humedad y la capacidad de campo de las muestras de suelo sin enmendar y enmendado con GC2.

Se estudió la influencia de la concentración de prosulfocarb presente en el suelo en la disipación del herbicida. Para ello el herbicida se añadió a muestras de suelo duplicadas (700 g) en un volumen de agua desionizada y esterilizada con una concentración adecuada para alcanzar una concentración en suelo de 4 mg kg^{-1} (dosis agronómica) y de 10 mg kg^{-1} (2.5 veces la dosis agronómica). Posteriormente, se añadió agua desionizada suficiente para incrementar la humedad a un 40% de la capacidad de campo. Esta humedad se mantuvo constante durante todo el experimento mediante el aporte de pequeñas cantidades de agua desionizada para compensar las pérdidas. Las muestras se incubaron en recipientes de polipropileno a 20ºC en cámara termostatizada y en condiciones de oscuridad.

Además, se prepararon muestras de 300 g de suelo esterilizadas utilizando una autoclave a una temperatura de 120 ºC durante 1 h tres días seguidos. Estas muestras se prepararon en matraces Erlenmeyer con tapón de algodón estéril envuelto en gasa y cubierto con papel de aluminio para minimizar las pérdidas de humedad. El herbicida se aplicó a estas muestras estériles alcanzando una concentración de 4 mg kg^{-1}. Estos suelos estériles se utilizaron como controles para comprobar la degradación química del herbicida. Todo el manejo de las muestras estériles se realizó en cámara de flujo laminar y todo el material de manipulación y muestreo fue esterilizado previamente con etanol.

Por otra parte, se realizó un experimento paralelo utilizando el compuesto marcado en ^{14}C. Se incubaron al mismo tiempo muestras por duplicado de suelo sin enmendar y enmendado con GC2 para estudiar el mecanismo de disipación de prosulfocarb y su biodisponibilidad. Un volumen de 10 mL de solución acuosa del herbicida no marcado se marcó con ^{14}C-prosulfocarb y se añadió a muestras de 500 g de suelo sin enmendar y enmendado con GC2 para alcanzar concentraciones de 4 mg kg^{-1} y 10 mg kg^{-1} en suelo seco y una actividad de aproximadamente 100 Bq g^{-1}. Al igual que con el herbicida no marcado, la humedad de las muestras se ajustó y mantuvo al 40% de la capacidad de campo durante todo el experimento. En los recipientes contenedores de estas muestras se dispuso un vial de centelleo con 1 mL de disolución acuosa de NaOH 1M mediante un clip de acero inoxidable a modo de trampa para retener el $^{14}CO_2$ (Reid et al., 2002).

Movilidad del herbicida

La movilidad del prosulfocarb se llevó a cabo utilizando columnas de suelo empaquetadas. Se utilizaron columnas de vidrio de 3 cm de diámetro interno y 25 cm de largo rellenas con 100 g de suelo sin enmendar o enmendado con GC2 en una proporción de 20% p/p. Estas columnas se saturaron con agua destilada introduciéndolas verticalmente en un recipiente con agua permitiendo que ascendiera por capilaridad. A continuación, se dejó drenar el exceso de agua durante 24 h para que la humedad fuera equivalente a la capacidad de campo. Se estimó el volumen de poro de las columnas de suelo por la diferencia de peso entre las columnas saturadas con agua y las columnas secas. El herbicida se añadió sobre la superficie de las columnas mediante la adición de 1 mL de una disolución en metanol de 1 mg mL^{-1} para alcanzar una concentración de 10 mg kg^{-1} en el suelo (2.5 veces la dosis agronómica) y una actividad aproximada de 10 kBq mL^{-1}.

La movilidad del herbicida se estudió en diferentes condiciones de lavado de las columnas y de envejecimiento del herbicida. Un grupo de columnas se lavaron inmediatamente después de la aplicación del herbicida mientras que en otro grupo el herbicida se incubó durante 28 días a 20 ºC en oscuridad para analizar la influencia de la incubación o envejecimiento del herbicida en el suelo sobre su movilidad. El lavado de las columnas se realizó con una disolución de $CaCl_2$ 0.01M en lugar de agua para minimizar la disrupción del balance mineral del suelo (Figura 10).

Se estudiaron dos flujos diferentes de lavado: flujo saturado y flujo saturado-no saturado. Bajo flujo saturado, se bombearon 500 mL de la disolución de $CaCl_2$ de manera continua a una velocidad de 1 mL min^{-1} manteniendo siempre la superficie de la columna en contacto con la disolución. Bajo flujo saturado-no saturado, se bombeó el mismo volumen total de disolución de $CaCl_2$ pero distribuido a lo largo de 25 días (20 mL / día). Para medir la mineralización del herbicida se dispuso una trampa en la parte alta de la columna con 1 mL de una disolución de NaOH 1M para atrapar el $^{14}CO_2$, de manera similar al indicado en el estudio de disipación en laboratorio. Para mantener un flujo constante de lavado se utilizó una bomba peristáltica modelo Gilson MINIPULS 3 (Gilson Inc. Middleton, WI, Estados Unidos). Las fracciones lixiviadas de 20 mL se recogieron con un colector automático de fracciones modelo Gilson F203. Una vez finalizado el lavado de las columnas, éstas se cortaron en tres segmentos (0-5 cm, 5-10 cm y 10-15 cm), se secó el suelo y se pesó para su posterior análisis.

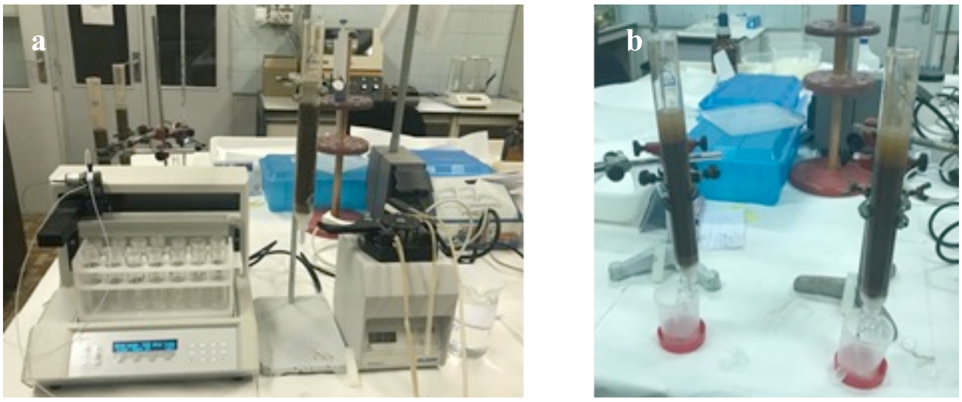


Figura10. Columnas empaquetadas y lavadas bajo flujo saturado (a) y flujo saturado-no saturado (b).

Extracción del herbicida y determinación analítica

Para la determinación del herbicida no marcado en el estudio de disipación se tomaron muestras de suelo por duplicado a diferentes tiempos. Los métodos utilizados para la extracción del herbicida desde las muestras de suelo y para su determinación mediante HPLC-MS son los indicados anteriormente para los experimentos de campo (Apartado 2.2.4).

El ^{14}C-prosulfocarb se extrajo de las muestras de suelo mediante un proceso secuencial. En primer lugar, se extrajeron 5 g de suelo por duplicado con 10 mL de solución acuosa de $CaCl_2$ 0.01 M mediante agitación durante 24 h. A continuación, se realizó una segunda extracción con 10 mL de metanol mediante agitación durante 24 h.

La determinación del ^{14}C-prosulfocarb se llevó a cabo mediante la medida de la actividad del ^{14}C de las soluciones en un contador de centelleo líquido modelo Beckman LS 6500 (Beckman Instruments Inc., Fullerton, CA) (Figura 11). La actividad del compuesto se midió en desintegraciones por minuto (dpm) y se llevó a cabo por duplicado tomando 1 mL de extracto en un vial de centelleo y añadiendo 4 mL de líquido de centelleo (Ecoscint TMA, National Diagnostics, Atlanta, GA). Se preparó un blanco por duplicado para cada suelo (utilizando agua como control) para medir las dpm que corresponden al fondo y corregir así interferencias que podría distorsionar la medida debido a los extractos solubles de los suelos. La determinación cuantitativa de la mineralización de prosulfocarb se realizó añadiendo 4 mL de líquido de centelleo al vial que contenía la disolución de NaOH 1M y midiendo la actividad en contador de centelleo líquido.

Figura 11. Contador de centelleo líquido.

Finalmente, se determinó el ^{14}C residual en el suelo a partir de la combustión de la muestra. La actividad remanente en las muestras debida al herbicida no extraíble o enlazado al suelo tras las extracciones se determinó por triplicado mediante combustión de 1 g de muestra previamente secada utilizando un horno de combustión Biological Oxidizer OX500 (R.J. Harvey Instrument Corporation, NJ, Estados Unidos) en corriente de oxígeno a 900 ºC (Figura 12).

Figura 12. Horno de combustión para muestras marcadas con ^{14}C.

El $^{14}CO_2$ generado fue atrapado en una mezcla de 1 mL de etanolamina y 15 mL de líquido de centelleo (Oxysolve C-400, Zinsser Analytic, Berkshire, Reino Unido) y analizado en contador de centelleo como se ha indicado anteriormente. Finalmente, se calculó el balance de masa de ^{14}C como porcentaje de la cantidad total de la actividad medida en las diferentes fracciones (líquidas y sólidas).

Determinación cuantitativa del ion trazador cloruro

Las características dispersivas de las columnas de suelo utilizadas en el estudio se describieron a partir del comportamiento de movilidad del ion trazador cloruro (Cl^-). Se añadieron 47 g de ion cloruro a cada columna correspondientes a 1 mL de una disolución de KCl de concentración 100 g L^{-1}. La movilidad del ion cloruro se estudió bajo los mismos flujos aplicados a las demás columnas (flujo saturado y flujo saturado-no saturado). La concentración del ion cloruro se determinó mediante cromatografía iónica utilizando un sistema modular de cromatografía iónica Metrohm (Metrohm Ltd., Herisau, Suiza) con supresión química (Figura 13).

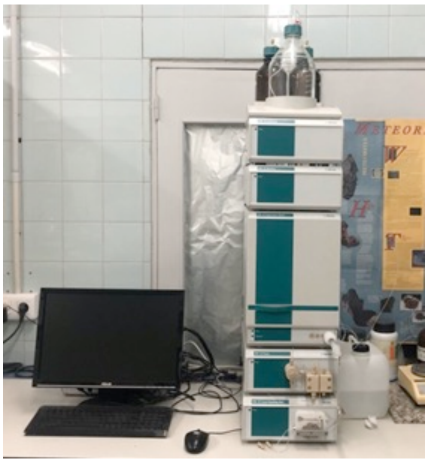


Figura 13. Cromatógrafo iónico para la determinación del ion trazador.

Este sistema está compuesto por un módulo de bombeo para el eluyente (Metrohm 709 IC Pump), una bomba peristáltica para el bombeo de las disoluciones de lavado y regeneración del supresor (Metrohm 752 Pump Unit), el centro de separación, donde se encuentra la columna y el módulo supresor (Metrohm 733 IC Separation Center), y el detector de conductividad (Metrohm 732 IC Detector). El sistema de adquisición y procesamiento de datos que se utilizó fue el IC Metrodata 714 para Windows 95. La columna usada fue 6.1006.520 Metrosep A Supp5 (Metrohm), formada por alcohol polivinílico con grupos amonio cuaternario, de un tamaño de 150 mm x 5 mm de diámetro interno y 5 μm de tamaño de partícula. La fase móvil estaba compuesta por una disolución en agua ultrapura Milli-Q (Millipore) con 3.2 mmol L^{-1} de Na_2CO_3, 1 mmol L^{-1} de $NaHCO_3$ y 100 mL de acetona. Se utilizó agua ultrapura Milli-Q (Millipore) y una disolución acuosa de ácido sulfúrico 20 mM como soluciones de lavado y de regeneración del supresor, respectivamente. La velocidad de flujo del equipo se fijó en 0.6 mL min^{-1} y el volumen de inyección fue 100 μL. Las muestras previamente filtradas a través de filtros GHP acrodiscs (Waters Corporation) de 0.2 μm de tamaño de poro, se inyectaron de modo manual.

3.2.7. Análisis de los resultados obtenidos

Las curvas de disipación para los herbicidas se ajustaron a dos modelos cinéticos (cinética de primer orden (SFO) y cinética de primer orden multicompartimental (FOMC)) utilizando la herramienta Solver del software Excel de Microsoft Office 2016. Se siguieron las recomendaciones del grupo de trabajo FOCUS (FOCUS, 2016) para elegir el modelo cinético que mejor describía los resultados experimentales. Se calculó el coeficiente de determinación (r^2) y la bondad de ajuste mediante el test de chi-cuadrado (χ^2). Para validar el modelo, los valores de χ^2 calculados deben ser iguales o menores al 15% para un número determinado de grados de libertad y un nivel de significación del 5%. Se calcularon los valores de vida media (DT_{50}) para caracterizar las curvas de disipación y comparar las variaciones en las velocidades de disipación para los diferentes tratamientos.

En algunos casos, la degradación de los herbicidas puede presentar una etapa inicial en la que la concentración inicial se mantenga constante durante un periodo determinado de tiempo, seguida de una etapa marcada por un descenso en la concentración de herbicida siguiendo un modelo monofásico o bifásico. Esta etapa inicial se denomina fase *lag* o de latencia y es debida al tiempo que necesitan los microorganismos para adaptarse al medio y poder comenzar a degradar el herbicida. Cuando esta fase *lag* está presente, el tiempo de vida media real del herbicida sería la suma de la fase inicial y del valor de DT_{50} calculado en la segunda fase.

Se realizaron análisis de varianza (ANOVA) para evaluar los efectos de los diferentes tratamientos en la disipación y movilidad de los herbicidas en los experimentos de campo y de laboratorio. La desviación estándar (SD) fue usada como indicador de la variabilidad entre réplicas de un mismo tratamiento. La diferencia mínima significativa (LSD) a un intervalo de confianza del 95% fue determinada para evaluar los efectos de los diferentes tratamientos en los tiempos de vida media de los herbicidas o en su persistencia en el perfil del suelo. En los estudios de la actividad deshidrogenasa y del perfil de ácidos grasos se utilizó el test post-hoc de Games-Howell a un valor de $p < 0.05$ para determinar diferencias significativas entre valores medios y evaluar los efectos de los diferentes tratamientos tanto a un tiempo de muestreo determinado como para todos los tiempos dentro de un mismo tratamiento. La relación entre la estructura y actividad microbianas y el porcentaje remanente de herbicida y humedad del suelo se determinó mediante los coeficientes de correlación de Pearson. Se realizaron análisis de componentes principales (PCA) para determinar las variables más influyentes y el impacto global de los herbicidas y compost vegetal sobre las comunidades microbianas del suelo. Estos análisis se llevaron a cabo utilizando el software IBM SPSS Statistics v24 (SPSS Inc. Chicago, Estados Unidos) y Past v3.15.

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RESULTADOS Y DISCUSIÓN

CAPÍTULO 1

Resumen

CAPÍTULO 1: Application of green compost as amendment in an agricultural soil: effect on the behaviour of triasulfuron and prosulfocarb under field conditions

RESUMEN

Los herbicidas son compuestos esenciales en sistemas agrícolas para mantener el rendimiento de los cultivos, ya que permiten controlar la aparición y/o crecimiento de malas hierbas que dan lugar a pérdidas en las cosechas. Por otra parte, la aplicación de residuos orgánicos al suelo como enmiendas se considera hoy día una práctica agrícola de interés ya que contribuye a aumentar el contenido en materia orgánica (MO) del suelo evitando su degradación. El empobrecimiento del suelo en MO es cada vez más evidente lo que hace necesario actuar para evitar la pérdida de fertilidad. Sin embargo, la aplicación de enmiendas orgánicas al suelo puede modificar el comportamiento de los herbicidas aplicados simultáneamente, influyendo en la efectividad de estos compuestos frente a las malas hierbas o incluso, de forma no prevista, en su persistencia o movilidad en el suelo dando lugar a problemas de contaminación ambiental.

En este trabajo se ha llevado a cabo un experimento de campo para estudiar la disipación, persistencia y movilidad de dos herbicidas (triasulfuron y prosulfocarb) aplicados en un suelo franco arcillo-arenoso como formulaciones comerciales individuales, Logran® (triasulfuron) y Auros® (prosulfocarb), o conjunta, Auros Plus®. Se estudió en un suelo sin enmendar y enmendado con compost vegetal (GC), residuo procedente de la poda de plantas en parques y jardines. Para realizar el experimento se establecieron parcelas experimentales de 9 m^2 de superficie en la finca experimental Muñovela del Instituto de Recursos Naturales y Agrobiología de Salamanca (IRNASA-CSIC). Se diseñaron, de manera aleatoria, parcelas sin enmendar y enmendadas con GC a la dosis de 120 t ha^{-1} en peso seco, y se dividieron en parcelas sin tratar con herbicidas y tratadas con las formulaciones comerciales Logran®, Auros® y Auros Plus® a las dosis de 4.5 kg i.a. ha^{-1} y 100 g i.a. ha^{-1} de prosulfocarb y triasulfuron, respectivamente. El experimento en campo tuvo una duración de 100 días.

Los resultados de disipación de los herbicidas mostraron que la aplicación de GC aumentó el tiempo de vida media (DT_{50}) del triasulfuron (46.7 días) respecto al suelo sin enmendar (19.4 días) cuando se aplicó como formulación individual. Este incremento fue debido a una mayor adsorción del herbicida por el suelo enmendado (con mayor contenido en MO) y no se observó cuando el triasulfuron se aplicó como una formulación

conjunta con prosulfocarb. Los coeficientes de adsorción (K_d) del triasulfuron por el suelo cuando se aplicó como formulación individual o conjunta indicaron un efecto competitivo por los lugares de adsorción. Estos coeficientes podrían explicar el aumento en su persistencia en suelos sin enmendar (aumento de K_d) y la disminución de su persistencia en el suelo enmendado (disminución de K_d) cuando se aplicó la formulación conjunta en comparación con la formulación individual. El herbicida prosulfocarb presentó una velocidad de disipación mayor que triasulfuron en todas las condiciones estudiadas. Los valores de DT_{50} variaron entre 13.9 y 18.5 días en suelos sin enmendar y enmendados aplicado como formulación individual o conjunta. A pesar del aumento de adsorción de prosulfocarb por el suelo enmendado, no se encontraron diferencias significativas entre las diferentes condiciones estudiadas probablemente debido a que la disipación de prosulfocarb, más hidrofóbico que triasulfuron, estaría controlada por una adsorción irreversible por el suelo independientemente del tratamiento.

El estudio de la movilidad de los herbicidas a diferentes profundidades en el perfil del suelo (0-50 cm) puso de manifiesto la lixiviación de ambos herbicidas a capas más profundas del suelo en todos los tratamientos, aunque la mayor cantidad de residuos se detectaron siempre en el tramo 0-10 cm. Se observó la presencia de concentraciones relevantes de triasulfuron en el tramo 10-20 cm en todos los tratamientos estudiados, y de pequeñas concentraciones de prosulfocarb en el suelo modificado con GC. La aplicación de la enmienda orgánica aumentó la persistencia de ambos herbicidas provocando un descenso de la lixiviación de triasulfuron cuando se aplicó como una formulación individual, pero no cuando se aplicó como una formulación conjunta con prosulfocarb, movilizándose en ese caso hasta una profundidad de 40-50 cm. La lixiviación del prosulfocarb no se vio inhibida por el GC detectándose el compuesto a lo largo de todo el perfil del suelo a 100 días después de su aplicación tanto en el suelo sin enmendar como enmendado. La movilidad del prosulfocarb no se vio afectada por el tipo de formulación aplicada a pesar de que los coeficientes de partición K_d cuando se aplicó bajo la formulación conjunta con triasulfuron en el suelo enmendado (21.6 g mL^{-1}) y en el suelo sin enmendar (30.7 g mL^{-1}) disminuyeron en comparación a los valores observados para la aplicación individual (24.1 y 37.2 g mL^{-1}, respectivamente). La movilidad de ambos herbicidas en todo el perfil del suelo podría haber estado influenciada por el carbono orgánico disuelto (COD) derivado del GC. Los resultados mostraron además la estrecha relación entre el contenido de agua en el perfil del suelo y la lixiviación del triasulfuron, pero no la del prosulfocarb.

Estos resultados resaltan el interés de obtener datos de campo para poder optimizar la aplicación conjunta de GC y herbicidas, y así prevenir la disminución de su efectividad contra las malas hierbas y el riesgo de contaminación de las aguas.

Artículo

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Research article

Application of green compost as amendment in an agricultural soil: Effect on the behaviour of triasulfuron and prosulfocarb under field conditions

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Herbicides are essential in agricultural systems for maintaining crop yields, as weeds compromise grain production. Furthermore, the application of organic amendments to soil is an increasingly frequent agricultural practice for avoiding irreversible soil degradation. However, this practice could modify the behaviour of the herbicides applied, with implications for their absorption by weeds. This study evaluated the dissipation, persistence and mobility of the herbicides triasulfuron and prosulfocarb in a sandy clay loam soil unamended and amended with green compost (GC) in a field experiment using single or combined commercial formulations of both herbicides. The study was carried out in experimental plots (eight treatments × three replicates) corresponding to unamended soil and soil amended with GC, untreated and treated with the herbicide formulations Logran®, Auros® and Auros Plus® over 100 days.

The half-life (DT_{50}) of triasulfuron applied individually was 19.4 days, and increased in the GC-amended soil (46.7 days) due to its higher adsorption by this soil, although non-significant differences between DT_{50} values were found when it was applied in combination with prosulfocarb. Prosulfocarb dissipated faster than triasulfuron under all the conditions assayed, but non-significant differences were observed for the different treatments. The analysis of the herbicides at different soil depths (0–50 cm) after their application confirmed the leaching of both herbicides to deeper soil layers under all conditions, although larger amounts of residues were found in the 0–10 and 10–20 cm layers. The application of GC to the soil increased the persistence of both herbicides, and prevented the rapid leaching of triasulfuron in the soil, but the leaching of prosulfocarb was not inhibited. The influence of single or combined formulations was observed for triasulfuron, but not for prosulfocarb. The results obtained highlight the interest of obtaining field data to design rational joint applications of GC and herbicides to prevent the possible decrease in their effectiveness for weeds or the risk of water contamination.

1. Introduction

Herbicides are essential in agriculture systems worldwide for protecting crops against weeds. Besides, the application of organic amendments to soil is an increasingly frequent agricultural practice in Spain for avoiding irreversible soil degradation. These materials increase the amount of organic matter (OM) in the soil, which plays a key role in the development and functioning of terrestrial ecosystems (García Izquierdo and Lobo Bedmar, 2008) and have attracted considerable interest in recent years (Scotti et al., 2015). In Spain, rising potentially exploitable organic wastes includes biodegradable wastes generated by pruning in parks and gardens. These residues referred to as green compost (GC) become materials with OM > 15%, and they could be used as organic amendments.

However, this practice could lead to changes in the behaviour of herbicides applied in amended soils (Barriuso et al., 1997). The herbicides could be adsorbed by amended soils, and this could have consequences for their degradation, persistence or mobility (Marín-Benito et al., 2012, 2013; Rodríguez-Liébana et al., 2014; Keren et al., 2017). These processes could decrease pollution by

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herbicides, but they could also affect the final concentration bioavailable for their absorption by the targeted weeds (Barriuso et al., 1997). These effects are generally studied in the laboratory, with fewer studies being carried out under real field conditions (Herrero-Hernández et al., 2011, 2015).

Triasulfuron and prosulfocarb are two herbicides used in pre- and post-emergence against weeds in winter cereals (wheat and barley) and other crops (potato). Triasulfuron is a sulfonylurea with high water solubility and a low hydrophobic nature (PPDB, 2017). This compound is a weak acid (pKa 4.8) found predominantly as an anion at high pH and thus being more available for plants, while in acid soils it is less available since it is more adsorbed by soil (EC, 2000). In high pH soils, therefore, the rapid leaching of the herbicide and a deeper displacement of triasulfuron slowly degrading in the profile are to be expected because microbial activity usually decreases with depth. The influence of biotic (microbial activity) and abiotic (pH) factors on its persistence have been reported (Singh and Kulshrestha, 2006), as well as the influence of adsorption by soil on triasulfuron's biodegradation and bioavailability (Said-Pullicino et al., 2004).

The dissipation of triasulfuron herbicide has been studied in the laboratory and in the field (EC, 2000; Gennari et al., 2008), while only some laboratory studies have evaluated the effect of the herbicide's application dosage (Sofo et al., 2012; Pose-Juan et al., 2017) or the influence of OM on its adsorption and dissipation in amended soils (Said-Pullicino et al., 2004; Pinna et al., 2009; Pose-Juan et al., 2017). Evaluating the changes in mobility and the persistence of this herbicide by soil amendment is therefore of interest, as it could impact negatively on the performance of future crops (Menne and Berger, 2001; Sarmah et al., 1998, 2000). In fact, this herbicide has been reported to persist in soil at phytotoxic concentrations for more than a year after application, especially at low temperatures and high pH, causing damage to sensitive crops in subsequent rotations (James et al., 1999).

Prosulfocarb is a thiocarbamate non-ionic and hydrophobic compound (PPDB, 2017). Mean values of the time required for the concentration to decline to half of the initial value (DT_{50}) between 15.2 and 10.1 days were reported for different soil types under laboratory and field conditions, respectively (EFSA, 2007). The dissipation of prosulfocarb is due mainly to a microbial process, and Gennari et al. (2002) have reported that the degradation rate was inversely correlated with organic carbon (OC) content. Accordingly, it records high adsorption and persistence associated with all soil OM fractions (Braun et al., 2017) which lead to a decrease in leaching and plant bioavailability, and therefore in herbicide performance (Nègre et al., 2006).

Weed control in cereals with these herbicides is widely recommended, using them as the single formulations Logran® and Auros®, respectively, or as a combined formulation of both herbicides, Auros Plus® (De Liñán Vicente and De Liñán Carral, 2008). The application of Auros® is recommended as a combined formulation with other herbicides when a varied infestation occurs or for managing weeds' herbicide resistance (Busi and Powles, 2016). The studies in the literature generally evaluate the behaviour of individual molecules in unamended soils, but to our knowledge no one has addressed the dissipation of these herbicides applied in combination or in the presence of organic amendments. It has been reported that microbial activities could be affected by the presence of an herbicide in the soil, and this could affect the degradation, persistence and leaching of another herbicide applied at the same time (Fogg et al., 2003; Swarcewicz et al., 2013; dos Reis et al., 2017).

Accordingly, this study was carried out to evaluate the dissipation, persistence and mobility of the herbicides triasulfuron and prosulfocarb in a sandy clay loam soil. A field experiment was set up, and the effects of single or combined commercial formulations of these herbicides, until now not studied, on the dissipation of individual compounds was evaluated in an unamended soil and in a soil amended with GC.

2. Materials and methods

2.1. Herbicides

This study used the commercial formulations of triasulfuron (Logran® 20% p/p), prosulfocarb (Auros® 80% p/v) and triasulfuron + prosulfocarb (Auros Plus®) (Syngenta Agro S.A., Madrid, Spain). Analytical standards of both compounds were purchased from PESTANAL® (purity > 98.9%) (Sigma Aldrich Química S.A., Madrid, Spain). Water solubility is 815 and 13.0 mg L^{-1} and log Kow is −0.59 and 4.48 for triasulfuron and prosulfocarb, respectively. The chemical name, structure and specifications of these compounds are included in Table S1 in Supplementary material (PPDB, 2017).

2.2. Green compost

A composted organic residue of vegetal origin generated from the pruning of plants and trees in parks and gardens in the city of Salamanca (Spain) was used. It was supplied by the City Council. The physicochemical characteristics of this green compost (GC) on a dry weight basis are as follows: pH 7.33, determined in a GC/water suspension (1:2.5); organic carbon (OC) content 9.80%, determined by the modified Walkley-Black method; dissolved organic carbon (DOC) 0.353%, determined in a suspension of soil in deionized water (1:2) after shaking (24 h), centrifugation (20 min at 10000 rpm), and filtering using a Shimadzu 5050 organic carbon analyser (Shimadzu, Columbia, MD, USA); total N 1.04%, determined by the Kjeldahl method. The C/N ratio was 9.42, and the ash percentage determined by weight difference after ignition at 540 °C for 24 h was 74.5%. All parameters were determined by standard methods (Sparks, 1996).

2.3. Experimental set-up

The field experiment was conducted in an agricultural sandy clay loam soil (Typic Haploxerept) located in the Muñovela experimental farm belonging to the Institute of Natural Resources and Agrobiology of Salamanca, Spain (latitude 40°55′56″ N, longitude 5°52′53″ W). Rainfall and air temperature were monitored over the 100 days of experimentation at a meteorological station installed on site. An experimental layout of randomized complete blocks was designed in February 2015, with eight treatments and three replicates per treatment (24 plots of 3 m × 3 m) corresponding to unamended soil (12 plots) and soil amended with GC at the rate of 120 t ha^{-1} on a dry weight basis (~11.6 t C ha^{-1}) (12 plots) (Figure S1 in Supplementary material). Three unamended and three amended plots were treated with single commercial formulations of each herbicide (Logran® and Auros®), or a combined commercial formulation of both herbicides (Auros Plus®). Three more control plots (unamended and amended) did not receive any herbicide. Prior to amendment, the soil was tilled using a field cultivator, and then GC was manually mixed with the topsoil (0–20 cm) in each plot. The study was conducted in the cereal growing season, but the plots were left fallow.

2.4. Application of herbicides

Water-herbicide solutions were applied manually using a backpack sprayer (volume of 10 L) shortly after the soil was amended. The doses applied to the plots were 4.5 kg i.a. ha^{-1} as Auros® 80% and 100 g i.a. ha^{-1} as Logran® 20%, corresponding to the recommended application dose for prosulfocarb and double this dose for triasulfuron, respectively (De Liñán Vicente and De Liñán Carral, 2008). Similar doses of both compounds were applied jointly as Auros Plus®. The theoretical concentrations were 0.077 mg kg^{-1} and 3.46 mg kg^{-1} for triasulfuron and prosulfocarb, respectively, calculated considering a soil density of 1.3 g cm^{-3} and a depth of 10 cm, although the real concentrations measured ranged from 0.073-0.211 mg kg^{-1} and 4.56–5.22 mg kg^{-1} for both compounds, respectively, in different plots. A check was made prior to the application of the herbicides to ensure that no amounts of these compounds were detectable in the soil samples. This was as expected, because the plots had no history of triasulfuron and prosulfocarb application in the previous five years.

2.5. *Soil sampling and sample processing*

Soil samples from 0 to 10 cm were collected on the first day after herbicide application to determine the initial concentration of compounds, and after 2, 7, 9, 15, 29, 51 and 100 days after treatment to determine herbicide dissipation. Five sub-samples were taken in each plot, mixing them before they were transferred to polypropylene bottles. Additionally, five soil cores were also collected in each plot to a depth of 50 cm at 15, 30 and 100 days after application to study the herbicide's downward mobility in the soil. The cores were then sectioned into five segments of 10 cm each, and composite samples of five cores were transferred to a polypropylene bottle. All the samples were transported to the laboratory in portable refrigerators. The soil water content of the bulk sample for each 10-cm soil layer was gravimetrically determined by weight difference, measuring the soil sample mass before and after drying at 110 °C for 24 h. Bulk density was obtained in an additional core by calculating the weight of dry soil contained in the volume (31.4 cm^3) of a 10-cm sampling. It ranged from 1.39 to 1.59 g cm^{-3} and from 1.0 to 1.59 g cm^{-3} in the soil profile for the unamended and amended soils, respectively. The profiles of the volumetric water content in plots with different treatments were determined from bulk density and gravimetric moisture content at 15, 30 and 100 days after herbicide application. Soil samples, previously air-dried overnight if necessary, were then sieved (<2 mm) and their characteristics determined by standard methods (Sparks, 1996) (Table 1). The pH of the soils was determined in soil/water suspensions (1:2.5), and OC and DOC were determined as previously indicated. Inorganic carbon was determined as $CaCO_3$ with a Bernard calcimeter. Particle size distribution was determined using the pipette method, and clay minerals were identified qualitatively by the X-ray diffraction technique using a Philips PW 1710 diffractometer (Eindhoven, the Netherlands) (Robert, 1975). The Rawls pedotransfer function (Rawls et al., 1982) was used to calculate the soil water content at field capacity (θ_{FC}, 33 kPa) for each 10-cm soil layer. These values vary from 0.264 to 0.294 cm^3 cm^{-3} in the unamended soil profiles, and from 0.267 to 0.359 cm^3 cm^{-3} in the amended soil profiles (Table 1).

2.6. *Herbicide extraction and analysis*

Duplicate subsamples of moist soil (6 g) from each plot were transferred to a glass tube, and extracted with methanol (12 mL). The samples were sonicated for 1 h, shaken at 20 °C for 24 h, and then centrifuged at 5045 g for 15 min. A volume of 8 mL was transferred to a clean glass tube and evaporated until dryness at 25 °C under a nitrogen stream using an EVA-EC2-L evaporator (VLM GmbH, Bielefeld, Germany). The residue was dissolved in 0.5 mL of methanol+formic acid (1%), filtered to remove particles > 0.45 μm in a GHP Acrodisc filter (Waters Corporation), and transferred to a HPLC glass vial for analysis.

The analysis of triasulfuron and prosulfocarb was performed by HPLC. The apparatus used was a Waters chromatograph (Waters Assoc., Milford, MA, USA), equipped with a model e2695 multisolvent delivery and autosampler system attached to a ZQ mass spectrometer detector (MS), with Empower software as the data acquisition and processing system. A Luna® 3 μm PFP(2) 100 Å (150 × 4.6 mm) column by Phenomenex (Torrance, CA, USA) was used at ambient temperature, and the mobile phase was acetonitrile:water+1% formic acid (70:30). The flow rate of the mobile phase was 0.4 mL min^{-1}, and the sample injection volume was 10 μL. Detection involved monitoring the positive molecular ion [m/z] 402.8 $[M+H]^+$ (triasulfuron) and 252.4 $[M+H]^+$ (prosulfocarb) after applying an optimized cone voltage of 20 V, and the retention times were 6.1 min and 14.1 min, respectively. The matrix-matched calibration standards were between 0.1 and 2.5 μg mL^{-1} for both herbicides, and the limit of detection (LOD) and limit of quantification (LOQ) were in the ranges 0.018–0.026 μg mL^{-1} or 0.059–0.088 μg mL^{-1} for triasulfuron, and 0.005–0.008 μg mL^{-1} or 0.017–0.027 μg mL^{-1} for prosulfocarb in the unamended and amended soils, respectively (Table S2 in Supplementary material). The method's recoveries were determined by spiking three unamended and amended soil samples with triasulfuron or prosulfocarb at similar concentrations to those applied in the field, and performing the extraction procedure as described above. The mean recovery values for a level spiking of 76.9 μg kg^{-1} of triasulfuron were >80% when triasulfuron was applied individually (Logran®) or in combined form (Auros Plus®) and for a level spiking of 3.46 mg kg^{-1} of prosulfocarb were >90% for prosulfocarb applied individually (Auros®) or >80% when it was applied in combined form (Auros Plus®) for the unamended and GC-amended soils (Table S2 in Supplementary material). These recoveries were considered acceptable according to Good Laboratory Practice (GLP) requirements for residues analysis (70–100% recovery) (Gennari et al., 2000). The amounts of triasulfuron and prosulfocarb extracted from soils were not corrected for recovery values because matrix-matched calibration method was used.

Furthermore, positive molecular ions (m/z) 141.15 $[M+H]^+$ (triazine amine), 388.8 $[M+H]^+$ (O-demethyl triasulfuron) and 267.4 $[M+H]^+$ (prosulfocarb sulfoxide) were qualitatively monitored for the detection of triasulfuron and prosulfocarb metabolites (EFSA, 2007; EFSA, 2015).

2.7. *Adsorption experiments*

The adsorption of triasulfuron and prosulfocarb by triplicate unamended and amended soils (5 g) was determined as a single-point concentration at the beginning of the experiment. Solutions of individual or combined compounds at a concentration of 10 mg L^{-1} (10 mL) were used. The suspensions were shaken intermittently at 2–3 h intervals for 24 h in a thermostated chamber at 20 °C. They were subsequently centrifuged at 5045 *g* for 15 min, and the herbicide concentrations were determined as previously indicated. The amount of herbicide sorbed (Cs, mg kg^{-1}) was considered the difference between that initially present in the solution (Ci, mg L^{-1}) and that remaining after equilibration with the soil (Ce, mg L^{-1}). Distribution coefficients, Kd (L kg^{-1}), were calculated from the relationship between Cs (mg kg^{-1}) and Ce (mg L^{-1}) ($Kd = Cs/Ce$).

2.8. *Data analysis*

The dissipation kinetics for both herbicides was fitted to a single first-order (SFO) kinetic model ($C = C_0\ e^{-kt}$) or first order

Table 1
Properties of the unamended soil and green compost amended soil from experimental plots.

Properties	Soil depth (cm)				
	0–10	10–20	20–30	30–40	40–50
Soil texture	Sandy clay loam	Sandy clay loam	Sandy clay loam	Sandy clay loam	Sandy clay
pH	7.81[a]	7.63	7.51	7.45	7.42
	7.13–7.39[b]	7.15–7.44	7.26–7.48	7.49–7.55	7.34–7.43
Bulk density (g cm^{-3})	1.39[a]	1.42	1.49	1.49	1.59
	1.00[b]	1.00	1.49	1.49	1.59
OC (%)	1.30[a]	1.13	0.86	0.70	0.50
	2.21–2.36[b]	1.25–1.69	0.90–0.93	0.75–0.79	0.53–0.54
DOC (%)	0.002[a]	–	–	–	–
	0.015–0.019[b]	–	–	–	–
N (%)	0.14[a]	0.11	0.09	0.08	0.07
	0.23–0.25[b]	0.13–0.14	0.10–0.10	0.08–0.08	0.07–0.07
C/N	9.28[a]	10.3	9.56	7.50	6.71
	9.44–9.61 [b]	9.62–12.1	9.30–11.3	9.38–9.88	7.71–7.57
$CaCO_3$ (%)	0.21[a]	0.18	0.20	0.22	0.22
Sand (%)	57.63[a]	58.22	59.15	55.63	51.04
Silt (%)	16.97[a]	16.95	16.95	16.77	17.60
Clay (%)	24.98[a]	25.25	25.25	29.10	34.72
Clay mineralogy	K, I, M[c]	K, I, M	K, I, M	K, I, M	K, I, M
θ_{FC} (cm^3 cm^{-3})[d]	0.291[a]	0.282	0.264	0.280	0.294
	0.359[b]	0.326	0.267	0.282	0.296
Soil water content (cm^3 cm^{-3})	0.117–0.167[a]	0.157–0.161	0.156–0.167	0.153–0.166	0.157–0.161
	0.085–0.128[b]	0.093–0.120	0.123–0.167	0.116–0.158	0.128–0.162

Note: The ranges of values included for some parameters correspond to the variation of mean values of these parameters in three replicate plots at different sampling times. DOC only was determined at 0–10 cm soil depth.

[a] Unamended soil.

[b] Soil amended with green compost at 120 t ha^{-1}.

[c] K, kaolinite; I, illite; M, montmorillonite.

[d] Volumetric water content at field capacity (33 kPa) estimated from Rawls et al. (1982).

multicompartment (FOMC) model ($C = C_0/(t/\beta) + 1)^{\alpha}$), known also as the Gustafson and Holden model. FOCUS work group guidance recommendations were followed (FOCUS, 2006) for selecting the kinetic model that best describes the dissipation results. The coefficient of determination (R^2) and the chi-square (χ^2) test were calculated as indicators of the goodness of fit. The χ^2 test considers the deviations between observed and calculated values relative to the uncertainty of the measurements for a specific fit, and was used to compare the goodness of fit of the two models tested. The error value at which the χ^2 test is fulfilled at a given degree of freedom should be below 15% (at a 5% significance level). Values for the time to 50% dissipation (DT_{50}) were used to characterize the decay curves and compare variations in dissipation rates. The parameters of the kinetic models were estimated using the Excel Solver add-in package (FOCUS, 2006).

Analysis of variance (ANOVA) was used to evaluate the effects of the different soil treatment on herbicide dissipation. Standard deviation (SD) was used to indicate variability among replicates. Fisher's least significant difference (LSD) method, at a confidence level of 95%, was determined with SPSS Statistics 22.0 software for Windows (SPSS Inc. Chicago, USA).

3. Results and discussion

3.1. Weather conditions and soil water content

Weather conditions were recorded throughout the experiment (100 days) (Fig. 1). After the application of herbicides, air temperature ranged from 1 °C to 22.7 °C during the time period. Cumulative precipitation during the experiment was 118.8 mm, but low-intensity rainfall occurred during the first 15 and 30 days (6.4 and 17.8 mm were recorded in total, respectively), corresponding to soil sampling and herbicide determination. Rainfall events >10 mm occurred at 36, 47, 89 and 91 days after herbicide application.

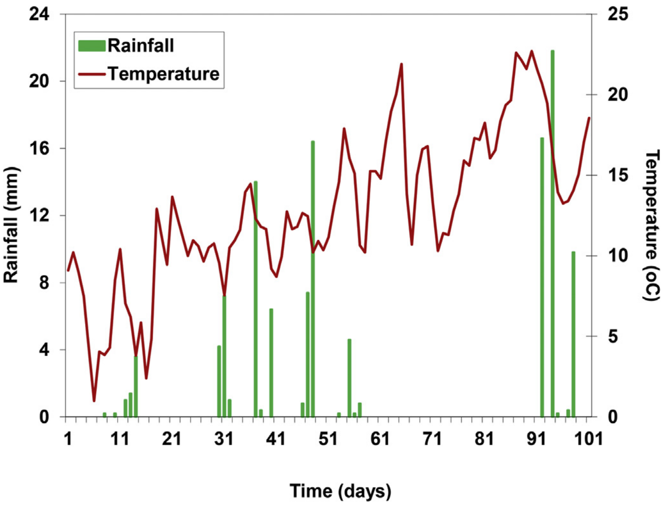

Fig. 1. Rainfall and temperature evolution over time of experiment.

Soil water content ranged between 0.100 and 0.174 cm^3 cm^{-3} (soil) and 0.079–0.132 cm^3 cm^{-3} (soil+GM) in the replicated plots at 0–10 cm depth, and increased to 0.142–0.178 cm^3 cm^{-3} (soil) and 0.123–0.164 cm^3 cm^{-3} (soil+GM) at 40–50 cm at different sampling times. The standard deviation of the means at each depth and replicate plots was <20%, indicating close agreement between plots treated with each formulation (Fig. 2). The highest values of water content in the soil profile were, in general, observed after 15 days of treatment in the unamended plots, and after 30 days in the amended plots. On the whole, soil water content increased sharply at 20 and 30 cm depth in the unamended and amended plots at the different sampling times, respectively. This increase was more pronounced in the amended plots. Both unamended and amended soil profiles recorded the highest degree of soil saturation at

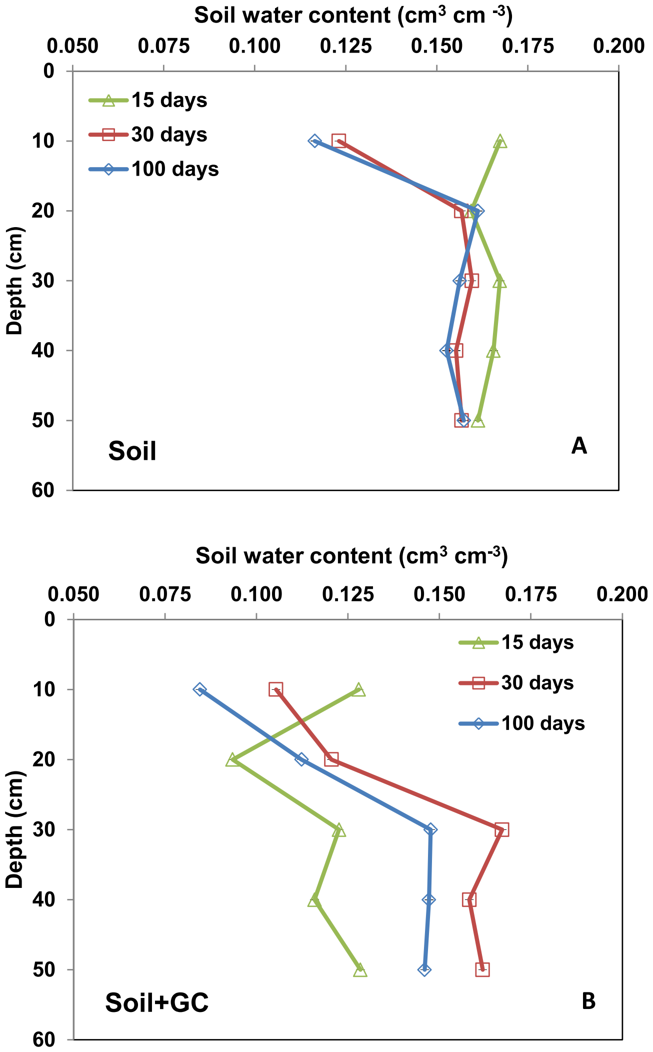

Fig. 2. Mean values of soil water content of the soil profile corresponding to plots of unamended soil (A) and green compost amended soil (B) at different sampling times. Error bars represent the standard deviation of the mean values of plots treated with different herbicide formulations (n = 3), note that they are smaller than the symbol.

20–30 cm depth. The saturation rate of this soil layer ranged from 59% to 63% and from 46% to 63% of the soil field capacity for the unamended and GC-amended soils at the different sampling times, respectively. The application of GC to soil increased the water-holding capacity of the 20-cm topsoil layers by between 16% and 23% (Table 1), which involved a decrease in the saturation rate of the 20-cm topsoil layers of between 13% and 28% at the different sampling times. Changes in GC-soil water content were initially more uneven (Fig. 2), and the distribution of water in the soil profile is more likely linked to light preferential flows in the soil than in the GC-soil, at least initially. Some authors have highlighted the ability organic amendments have to improve the soil's aggregation and structural stability, and its overall porosity, as well as modify pore size and connectivity, and consequently reduce the presence of preferential flows (Ingelmo Sánchez and Rubio Delgado, 2008; Yazdanpanah et al., 2016).

3.2. Dissipation of herbicides

The dissipation kinetics of triasulfuron and prosulfocarb in the unamended and GC-amended soils was determined from the herbicide concentrations in soil extracts (Figs. 3 and 4). These amounts were obtained at different times until 100 days after the application of herbicides. They were expressed as percentages of the amounts found in the samples taken immediately after application, which were considered a reference for the herbicide applied. Herbicide concentrations were fitted to kinetic equations, and DT_{50} values were calculated for triasulfuron and prosulfocarb for each one of the conditions established.

3.2.1. Triasulfuron dissipation

Triasulfuron dissipation followed slow and prolonged dissipation kinetics until 100 days (Fig. 3). In general, the dissipation kinetics of this herbicide in the unamended and GC-amended soils fitted the single first-order (SFO) kinetic model better than the first-order multi-compartment (FOMC) kinetic model (χ^2 values were lower than those corresponding to the FOMC model). The kinetics of triasulfuron dissipation fitted to a SFO model have been reported by some authors in laboratory studies, whereas in field studies the kinetics are better fitted to a biphasic model (Singh and Kulshrestha, 2006; EFSA, 2015). DT_{50} values were obtained from these equations for triasulfuron dissipation in each one of the plots (Table 2). The results obtained were very similar in some replicate plots (coefficient of variation (CV) < 10%), while they varied more in other plots (CV < 25%), as expected for a field experiment. This variability did not depend on the commercial formulation applied (Logran$^{®}$ or Auros Plus$^{®}$) or soil treatment. After 100 days of treatment, the residual amount of triasulfuron was <5% in the unamended soil and <10% in the amended one. These values indicate that the dissipation of the herbicide after 100 days of application was not complete, although it was slower in the amended soil than in the unamended one.

The DT_{50} mean value of triasulfuron in the unamended soil was 19.6 ± 2.17 days, and increased to 46.7 ± 11.6 days ($p < 0.05$) in the GC-amended soil when applied individually (Logran$^{®}$). However, non-significant differences were recorded between the DT_{50} of triasulfuron after its combined application with prosulfocarb (Auros Plus$^{®}$) in the unamended soil (24.5 ± 4.62 days) and in the amended one (24.5 ± 1.48 days), although a lag phase was observed in the unamended soil prior to herbicide dissipation when applied as Auros Plus$^{®}$. This phase, corresponding to the period of adaptation that the microorganisms undergo before they begin to degrade the compound, has been observed for other compounds (Marín-Benito et al., 2014).

The DT_{50} values found in this study are within the range observed in other field studies on unamended soils (<50 days) (Sarmah et al., 2000; EFSA, 2015), but they are lower than those obtained in laboratory experiments (33–76 days) (James et al., 1999; EC, 2000; Pose-Juan et al., 2017). Mobility into deeper layers was one the factors proposed for trisulfuron increased dissipation in the field. Triasulfuron is a weak organic acid, and the variable dissipation data could be explained by the influence of pH and other environmental factors, such as OM content or water content, in this herbicide's availability and dissipation (Pinna et al., 2009). On the other hand, triasulfuron degradation may be expected, although the transformation products described, such as triazine amine or demethyl triasulfuron (EFSA, 2015), were not found here.

The results indicating the increased DT_{50} values of triasulfuron in the GC-amended soil are in agreement with the adsorption coefficients K_d of triasulfuron for the soil+GC (0.53 mL g^{-1}) (Table S3, in Supplementary material), and higher than in the unamended soil (0.05 mL g^{-1}). An increased adsorption of this herbicide by soils amended with different composted organic residues has also been reported in laboratory studies (Said-Pullicino et al., 2004; Pose-Juan et al., 2017). However, the addition of straw residues as

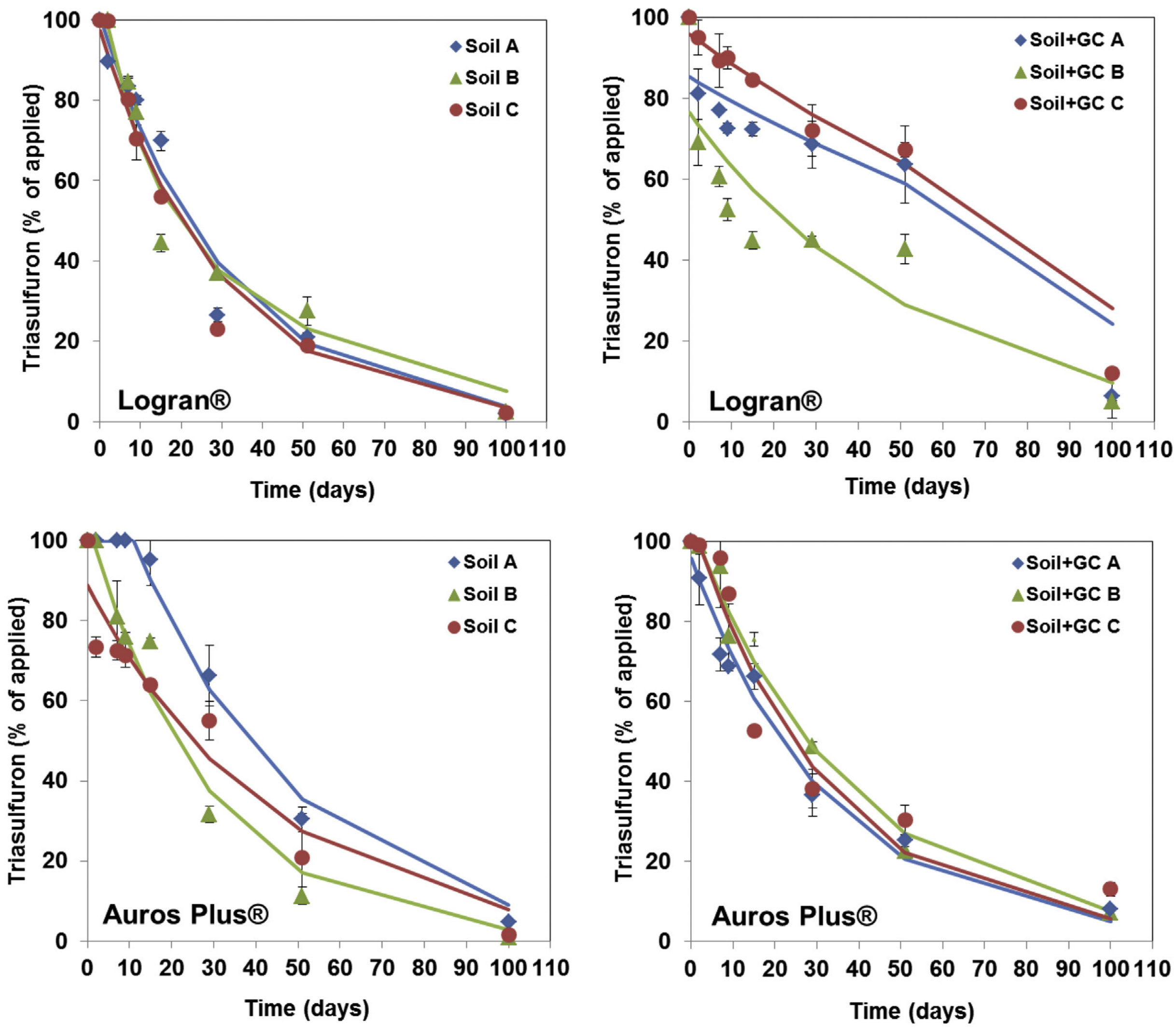

Fig. 3. Dissipation of triasulfuron applied as single formulation (Logran®) or combined formulation with prosulfocarb (Auros Plus®) in the replicate plots of unamended soil and green compost amended soil. Error bars represent the standard deviation of the mean values for each plot (n = 2).

organic amendment increased the dissipation of triasulfuron in lab experiments, but not in the field (Menne and Berger, 2001). The application of compost to the soil only influenced the dissipation of triasulfuron when it was applied individually, but not when applied as a combined formulation. Compost provides soils with OC and microorganisms that are capable of modifying the bioavailability of herbicides, possibly leading to increased or decreased dissipation (Barriuso et al., 1997). The results obtained could be explained by considering that in the presence of prosulfocarb (Table S3) the adsorption of triasulfuron by the unamended soil increased, and decreased for the amended soil (Kd = 0.31 and 0.38 mL g^{-1}, respectively). The combined application of herbicides could have a mutual influence on the behaviour of herbicides and on their own transformation rate (Menne and Berger, 2001). Both positive and negative effects due to the interactions of the compounds and microorganisms have been described, and may explain the results obtained when the combined formulation was applied (Fogg et al., 2003).

3.2.2. Prosulfocarb dissipation

Prosulfocarb followed biphasic kinetics; the first phase was rapid, and was followed by a slow and prolonged phase until 100 days (Fig. 4). These biphasic dissipations have frequently been observed for pesticides with different hydrophobic character (Rodríguez-Liébana et al., 2017). The dissipation kinetics of this herbicide fitted a SFO kinetic model in the unamended soil, and in general fitted a FOMC kinetic model in the GC-amended soil (Table 2) according to χ^2 values. The fitting of prosulfocarb dissipation in the unamended soil to a SFO model has also been reported by laboratory and field experiments (Rouchaud et al., 1997; Gennari et al., 1998; EFSA, 2007). The concentrations of herbicide decreased continuously in the second-phase until the last sampling after 100 days of treatment. Almost complete dissipation occurred in both the unamended and amended soils at this time, but there were still detectable amounts. A minimum residual concentration of prosulfocarb was found in the topsoil for all treatments (<0.5% in the unamended soil and <2% in the amended soil).

The dissipation of prosulfocarb was more rapid than that of triasulfuron, and the DT_{50} values of prosulfocarb ranged between 14.6 ± 3.06 to 18.5 ± 5.59 days in the unamended soil and between 14.2 ± 5.59 to 13.9 ± 3.10 days in the amended soil when a single or combined application with triasulfuron was used (Table 2). The DT_{50} values obtained here are within the range of those found in field experiments in unamended soils (EFSA, 2007, range 6.3–38.4

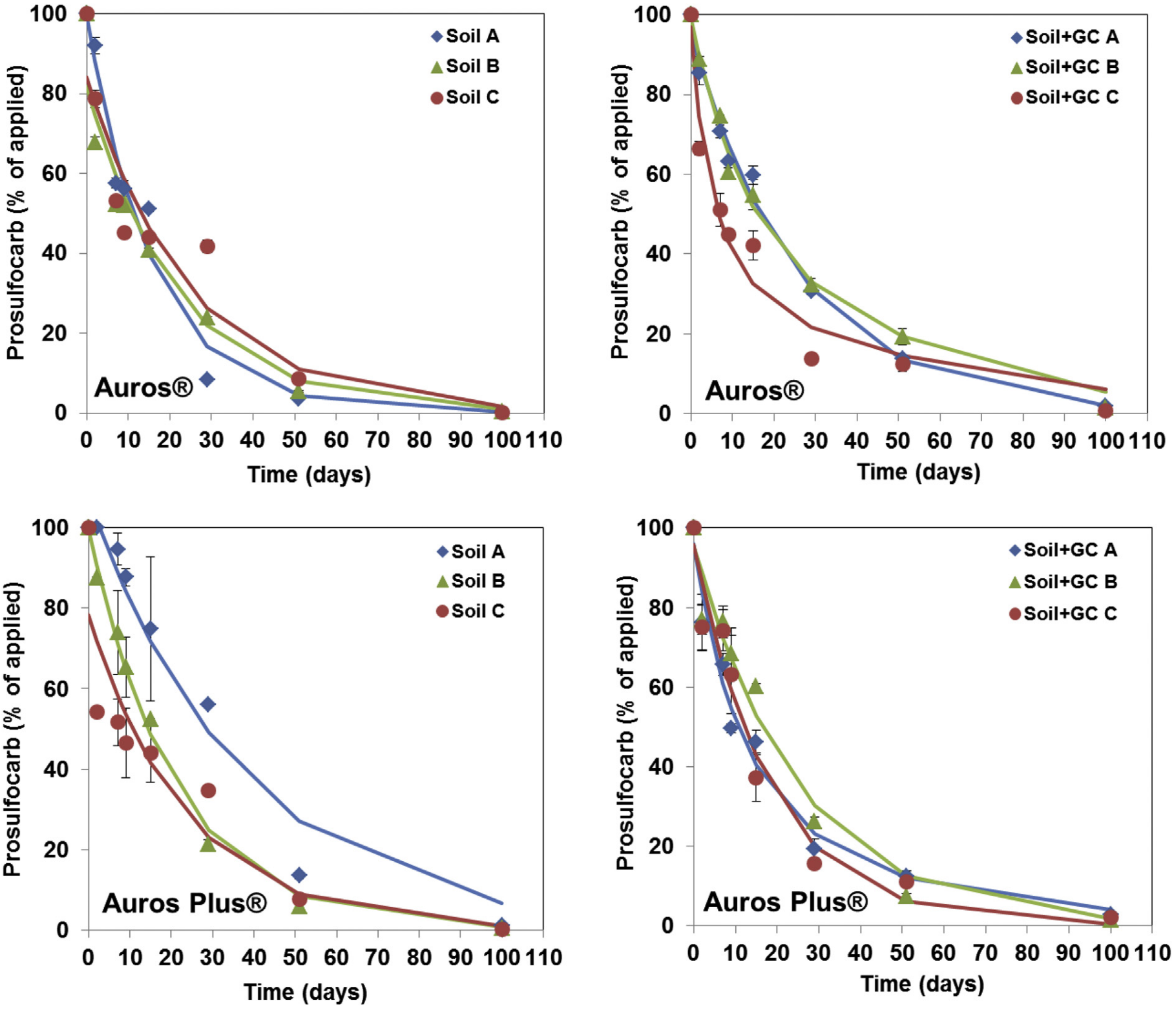

Fig. 4. Dissipation of prosulfocarb applied as single formulation (Auros®) or combined formulation with triasulfuron (Auros Plus®) in the replicate plots of unamended soil and green compost amended soil. Error bars represent the standard deviation of the mean values for each plot (n = 2).

days in soil with pH 4.8–7.5, and OC 0.7–3.1%). Gennari et al. (1998) have reported that the DT_{50} values of prosulfocarb in three soils are inversely related to soil OM content. However, this was not observed here, although the adsorption of prosulfocarb by the GC-amended soil was higher than that by the unamended soil, as indicated by the K_d values of 37.2 and 24.1 mL g^{-1}, respectively (Table S3). The results indicate that the dissipation rate was not significantly different for the three replicate plots, and non-significant differences were also observed between prosulfocarb dissipation in plots of unamended or amended soils when it was applied as Auros® or as Auros Plus®.

The rapid decrease in herbicide concentration in the surface soil observed in the first phase may be attributed to the transformation of the compound or bound residue formation in soils due to the high adsorption by soil OC (Gennari et al., 2002; Nègre et al., 2006; EFSA, 2007) or mobility in the soil profile. Some traces of prosulfocarb sulfoxide were detected in both the unamended and amended soils (data not shown), confirming the evidence reported on the microbial degradation of prosulfocarb (Gennari et al., 2002; EFSA, 2007). On the other hand, non-extractable bound residues could also form in the soil, as this has been considered the main cause of the dissipation of some hydrophobic compounds (Marín-Benito et al., 2014). However, the strongly adsorbed fraction of herbicide in the soil profile could also be mobile over time, as observed for other hydrophobic compounds in field dissipation studies (Herrero-Hernández et al., 2011, 2015).

3.3. Mobility of herbicides

The mobility of triasulfuron and prosulfocarb through the soil profile was verified in the unamended and GC-amended soil plots (Figs. 5 and 6) through the analysis of different soil depths (0–50 cm) after 15, 30 and 100 days of herbicide application.

3.3.1. Triasulfuron mobility

Triasulfuron mobility with the wetting front could be expected in the soil profile, as it has been reported that sulfonylureas adsorption decreases when soil pH increases (Pusino et al., 2003). This particularly occurs in soils with pH > pKa of compounds when they are in anionic form. The pH of the unamended and amended soils in the experimental plots was always >7 (Table 1), which means triasulfuron could be mobile with the wetting front of water because its pKa is 4.64. In this work, triasulfuron was mobile up to 50 cm, in agreement with other studies that have reported leaching and persistence of this herbicide in soils ranging from acid to slightly alkaline (Sarmah et al., 2000). The residual amount of

Table 2
Kinetic parameters for the dissipation of triasulfuron and prosulfocarb in unamended soil and green compost amended soil treated with individual or combined formulations of herbicides, obtained from fitting kinetics to a single first-order (SFO) and first order multicompartment (FOMC) models.

Herbicide (Formulation)	Plot	Soil			Soil+Green Compost		
		DT_{50} (days)	χ^2	R^2	DT_{50} (days)	χ^2	R^2
Trisulfuron (Logran®)	A	21.4^{a}	10.3	0.97	52.6^{a}	12.5	0.90
		20.7^{b}	8.6	0.98	52.6^{b}	13.3	0.90
	B	22.8^{a}	17.2	0.96	33.3^{a}	15.1	0.88
		16.9^{b}	9.1	0.97	33.2^{b}	19.3	0.87
	C	20.6^{a}	14.9	0.98	54.1^{a}	8.2	0.95
		20.6^{b}	20.1	0.98	54.1^{b}	8.8	0.95
	Mean ± SD	19.6 ± 2.17			46.7^{*} ± 11.6		
Trisulfuron (Auros Plus®)	A	$25.7^{a,c}$	6.5	0.99	23.5^{a}	5.8	0.99
		25.7^{b}	7.4	0.99	23.5^{b}	6.1	0.99
	B	19.4^{a}	7.9	0.99	26.2^{a}	5.2	0.99
		19.4^{b}	8.4	0.99	26.2^{b}	5.7	0.99
	C	28.4^{a}	10.4	0.96	23.8^{a}	10.1	0.97
		28.3^{b}	11.1	0.96	23.7^{b}	11.2	0.97
LSD (95%) 13.33	Mean ± SD	24.5 ± 4.62			24.5 ± 1.48		
Prosulfocarb (Auros®)	A	11.3^{a}	14.0	0.98	18.1^{a}	5.1	0.99
		15.2^{b}	38.4	0.98	18.1^{b}	5.5	0.99
	B	15.1^{a}	12.9	0.96	18.3^{a}	5.4	0.99
		15.3^{b}	23.2	0.96	16.7^{b}	4.8	0.99
	C	17.3^{a}	14.5	0.92	10.9^{a}	14.7	0.97
		22.1^{b}	28.5	0.91	7.8^{b}	12.1	0.98
	Mean ± SD	14.6 ± 3.06			14.2 ± 5.59		
Prosulfocarb (Auros Plus®)	A	24.9^{a}	8.2	0.98	13.2^{a}	9.2	0.98
		24.9^{b}	8.8	0.98	11.5^{b}	8.3	0.99
	B	14.4^{a}	3.9	0.99	17.4^{a}	8.8	0.98
		14.4^{b}	4.2	0.99	17.4^{b}	9.4	0.98
	C	16.3^{a}	14.4	0.91	12.8^{a}	10.4	0.98
		17.7^{b}	14.7	0.90	12.8^{b}	11.1	0.98
LSD (95%) 8.496	Mean ± SD	18.5 ± 5.59			13.9 ± 3.10		

[*] DT_{50} values marked with an asterisk indicate significant differences ($p < 0.05$).

[a] DT_{50} calculated from single first-order model (SFO).

[b] DT_{50} calculated from first order multicompartment model (FOMC).

[c] DT_{50} include a lag phase of 9 days.

triasulfuron applied as Logran® in the top layer of the unamended soil was 0.079 µg g^{-1} after 15 days of treatment (>50% of the amount initially determined and taken as application reference). Similar behaviour was observed after 30 days of treatment, although residual amounts decreased at all depths. At the end of the experiment, a significant decrease in herbicide residues was observed in all the soil profile, and an amount of 0.003 µg g^{-1} was found in the top layer. Triasulfuron mobility followed a similar pattern in both the GC-amended soil and in the unamended one, although the residual amounts after 15 days of treatment were lower than in the unamended soil, with 0.049 µg g^{-1} in the top layer. However, after 30 days of treatment the residual amounts increased at a depth of 30 cm, probably due to the sharp increase in the soil water content observed mainly for the amended soils at this depth, which would have facilitated the downwards movement of the herbicide (Fig. 2). The interaction in solution between triasulfuron and DOC derived from GC may decrease solute sorption and increase the mobility of this herbicide (highly soluble in water) in amended soils as reported for other pesticides (Álvarez-Martín et al., 2017; Marín-Benito et al., 2013; Rodríguez-Liébana et al., 2018). At the end of experiment, an amount of 0.006 µg g^{-1} was found in the top layer.

The herbicide concentrations on the soil surface decreased over time compared to those recorded immediately after its application, doing so to a greater extent in the unamended soil (43.2–97.8%) than in the amended one (32.8–92.1%). A lower adsorption of triasulfuron by the unamended soil (Table S3), together with a higher water saturation rate of the unamended soil profile previously indicated at this time (Table 1) could lead to a higher initial leaching of triasulfuron. An amount of 0.002 µg g^{-1} reached a depth of 30–40 cm after 15 days of application in the unamended soil, while the leaching of triasulfuron in the amended soil only reached this depth after 100 days of application. The water saturation rate of the GC-soil profile, which was higher at this time than at 15 days, could also explain this behaviour. The application of GC to the soil prevented the rapid leaching of triasulfuron, although it lasted longer in the topsoil and in the soil profile. Triasulfuron concentrations were found down to 30–40 cm (0.0003 µg g^{-1}) in the amended soil profile after 100 days of treatment, while residual amounts were detected only as far as 10–20 cm (0.0007 µg g^{-1}) in the unamended soil at this time. A low correlation was found between the adsorption constants of triasulfuron and the OC content of the soil profile ($p < 0.1$), although GC increased its adsorption in the top layer.

In spite of the clay texture of this soil, the anomalous peaks of the herbicide residues at deeper soil layers, which are indicators of preferential flows reported for other sulfonylureas, were not observed here (Sarmah et al., 2000). This means the presence of triasulfuron in the unamended soil profile in deeper layers at 15 days than at 100 days was not determined mainly by preferential flows, but agreed with the varying extent of water infiltration observed at both times.

The behaviour of triasulfuron applied as Auros Plus® followed a similar pattern, again recording the highest residual amounts in the top of the initially unamended soils (0.144 µg g^{-1}) (Fig. 5). Herbicide concentrations on the soil surface also decreased over time from those recorded immediately after its application. However, in this case, a greater decrease was generally observed in the amended soils (35.3–90.6%) than in the unamended ones (22.0–97.6%). The results indicate that the highest amounts of triasulfuron were

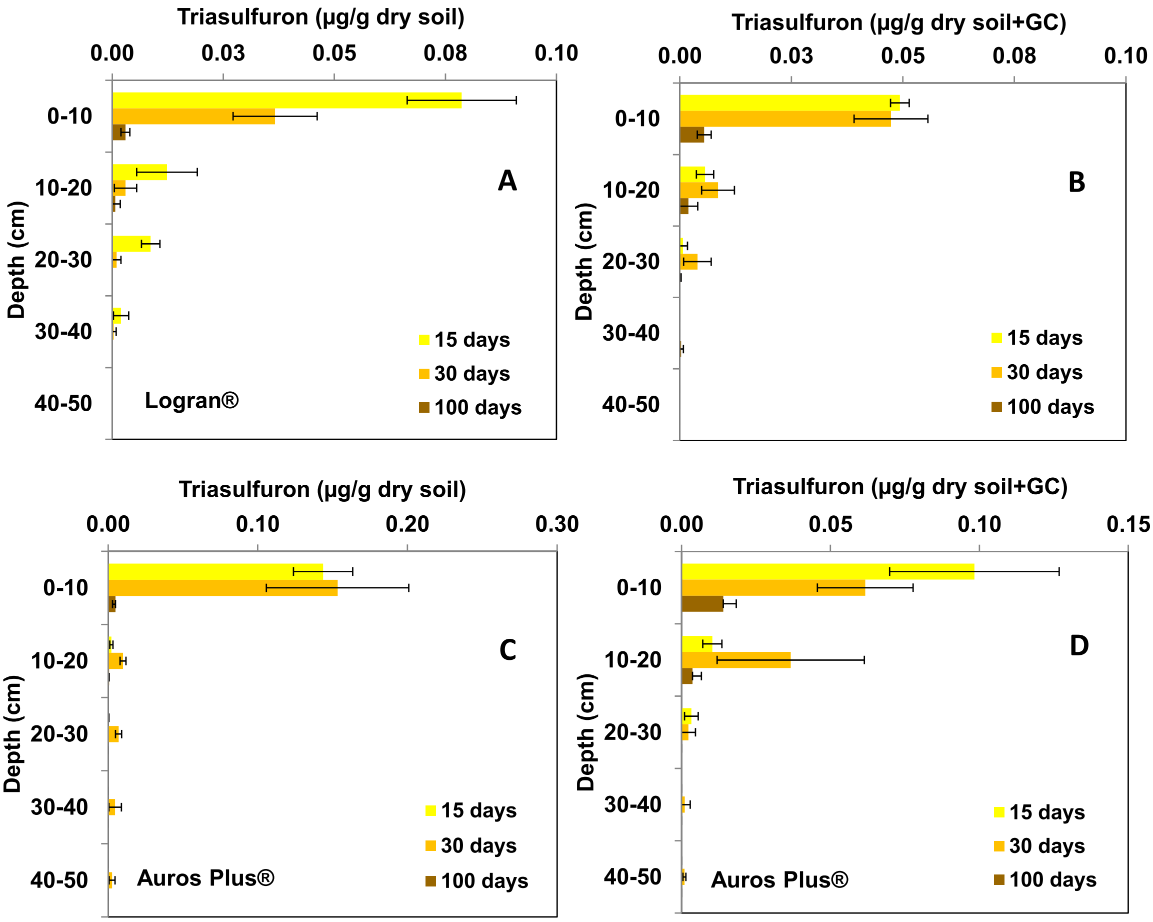

Fig. 5. Distribution profiles of triasulfuron applied as single formulation (Logran®) or combined formulation with prosulfocarb (Auros Plus®) in the replicate plots of unamended soil (A,C) and green compost amended soil (B,D) after different treatment times. Error bars represent the standard deviation of the mean values of plots treated with different herbicide formulations ($n = 3$).

leached after 30 days of application in both unamended and amended soils, when triasulfuron concentrations were found in the soil profile up to a depth of 40–50 cm in both the unamended soil (0.003 $\mu g\ g^{-1}$) and the amended one (0.001 $\mu g\ g^{-1}$). The adsorption coefficient K_d of triasulfuron together with prosulfocarb (Auros Plus®) by unamended soil increased, but decreased for the amended soil (Table S3) compared to the adsorption of the individual compound. This is consistent with the increased persistence of triasulfuron applied jointly with prosulfocarb in the unamended soil and its decreased persistence in the GC-amended soil compared to its single application. No significant correlation was found between the adsorption coefficients K_d and the OC content of the soil profile and the delayed leaching of triasulfuron until 30 days could be explained by a synergic effect of the mixed formulation. dos Reis et al. (2017) have reported a competitive effect for adsorption sites to explain the application method's influence on the leaching of certain herbicides.

In this field study changes in pH values were not observed and neither herbicide degradation was observed as reported in other studies (Walker and Welch, 1989). Other parameters, such as the different water saturation rates of the unamended and amended soil profiles, and the consequent different extent of water infiltration, might have contributed to the variable leaching behaviour of triasulfuron over time. It is remarkable that a rapid increase in soil water content was seen in depths of up to 30 cm after 30 days of herbicide application in the unamended soil, while the water content was initially less variable in the amended soil profile (Fig. 2). However, the presence of triasulfuron residues up to depths of 40–50 cm confirms the mobility of the herbicide down through the profile by increasing its water solubility.

3.3.2. *Prosulfocarb mobility*

The residual amount of prosulfocarb found in the unamended topsoil was 2.376 $\mu g\ g^{-1}$ after 15 days of its application as Auros® treatment, and it decreased to 0.018 $\mu g\ g^{-1}$ at the end of the experiment. In general, the amounts recorded were higher in the soils amended with GC (3.148 and 0.526 $\mu g\ g^{-1}$ after 15 or 100 days of application) than in the unamended one (Fig. 4). The highest prosulfocarb retention was recorded at a depth of 0–10 cm, and concentrations decreased over time compared to those recorded immediately after its application, doing so to a greater extent in the unamended soil (54.6–99.7%) than in the amended one (47.7–98.0%). The results agree with the adsorption coefficients K_d previously indicated (Table S3). However, a higher initial leaching of prosulfocarb was recorded in the amended soil, reaching a depth of 40–50 cm (0.029 $\mu g\ g^{-1}$) after 15 days of application, while in the unamended soil the herbicide reached down to 40–50 cm (0.101 $\mu g\ g^{-1}$) after 30 days of application. The GC soil amendment increased the persistence of prosulfocarb in the soil, but leaching was not inhibited, with this compound being detected through the soil profile up to 100 days after the application in both the unamended and amended soils (0.0024 and 0.0007 $\mu g\ g^{-1}$ were recorded at 40–50 cm, respectively). The water saturation rate of the unamended and amended soil profiles, which were the same as

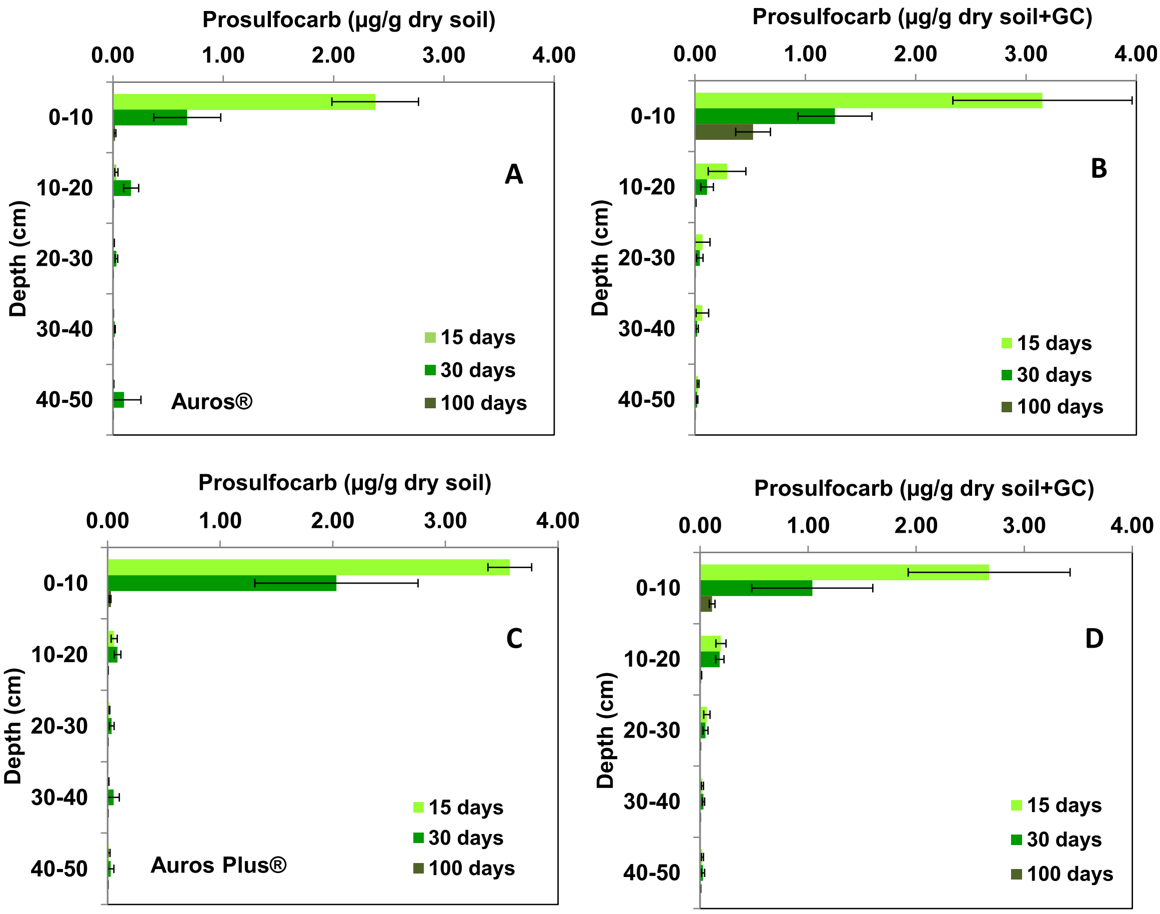

Fig. 6. Distribution profiles of prosulfocarb applied as single formulation (Auros®) or combined formulation with triasulfuron (Auros Plus®) in the replicate plots of unamended soil (A,C) and green compost amended soil (B,D) after different treatment times. Error bars represent the standard deviation of the mean values of plots treated with different herbicide formulations (n = 3).

for triasulfuron, do not explain the behaviour of prosulfocarb, which is a very hydrophobic compound. While, the DOC content of the amendment (range 0.015–0.019% over time) could affect the mobility of prosulfocarb in the amended soil profile. The adsorption of pesticides by DOC has been described in many papers in laboratory and field studies (Álvarez-Martín et al., 2017; Marín-Benito et al., 2013; Rodríguez-Liébana et al., 2018). This process has its effect on the mobility of pesticides in soils (Marín-Benito et al., 2016), and it could explain the leaching of prosulfocarb in spite of the rapid adsorption and/or dissipation in soil found here and in other papers (Braun et al., 2017) and its low-to-moderate potential for mobility in soil (EFSA, 2007). The results obtained show how GC initially increased herbicide retention on the surface, and a significant correlation was also found between the adsorption coefficients K_d of prosulfocarb by soil profile layers and each layer' OC content ($r = 0.99$, $p < 0.01$) (Nègre et al., 2006), although the herbicide's mobility could be enhanced over time at even deeper soil layers in amended soils.

The behaviour of prosulfocarb applied in combination with triasulfuron as Auros Plus® initially followed a different pattern to the single formulation with the highest residual amounts recorded in the unamended soils (Fig. 6). The herbicide concentrations in the soil surface decreased over time compared to those recorded immediately after application, but contrary to the Auros® treatment, it decreased more in the amended soil (52.1–97.9%) than in the unamended one (43.0–99.3%). The results indicate that the amounts of leached prosulfocarb obtained after 15 or 30 days of application were similar to those obtained when Auros® was applied, with the highest amounts leached after 30 days of application in both unamended and amended soils. Residues of prosulfocarb were found after 100 days at a depth of 50 cm at levels of 0.002 $\mu g\ g^{-1}$. The adsorption coefficients K_d of prosulfocarb as Auros Plus® for the unamended soil (21.6 g mL^{-1}) and the amended one (30.7 g mL^{-1}) decreased with respect to the K_d values of prosulfocarb as Auros®, but the herbicide's leaching behaviour was generally similar when applied as a single or combined formulation.

4. Conclusions

Our results show how GC decreases the dissipation of triasulfuron applied as an individual formulation due to the herbicide's increased adsorption in an amended soil. This effect was not seen when triasulfuron was applied as a combined formulation. The dissipation of prosulfocarb was more rapid than that of triasulfuron, and no significant differences were found under the different conditions studied. An irreversible adsorption of prosulfocarb by both unamended and amended soil could explain this result compared to triasulfuron, given the more hydrophobic nature of prosulfocarb. The mobility of herbicides through the soil profile was confirmed by the greater amounts of residues detected in the 0–10 cm layer for both herbicides and for all treatments at the different sampling times. However, relevant concentrations at 10–20 cm layers were also detected for triasulfuron under all conditions, with small concentrations for prosulfocarb in the GC-amended soil. GC decreased the leaching of triasulfuron applied as a single formulation, but not when applied as a combined formulation with prosulfocarb. No effect was observed for the single or combined formulations of prosulfocarb. The extent of triasulfuron leaching was closely linked to the extent of water infiltration, but this was not the case for prosulfocarb. The mobility of both herbicides throughout the soil profile might have been influenced by DOC derived from GC. The interest of these results is sustained considering leaching of herbicides from the soil surface shortens the duration of residual weed control, and may contaminate groundwater. On the other hand, the amounts retained in the soil profile could exceed the recorded threshold for the sensitivities of susceptible species. Further studies for a longer period are required to establish the influence of new agricultural practices in herbicide behaviour in order to improve their efficacy and environmental impact.

Conflicts of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jenvman.2017.11.024.

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Material Suplementario

SUPPLEMENTARY MATERIAL

APPLICATION OF GREEN COMPOST AS AMENDMENT IN AN AGRICULTURAL SOIL: EFFECT ON THE BEHAVIOUR OF TRIASULFURON AND PROSULFOCARB UNDER FIELD CONDITIONS

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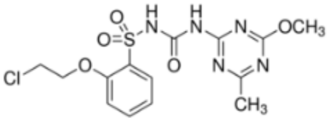
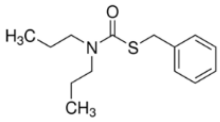
Nº Tables: 3

Nº Figures: 1

Table S1

Characteristics of herbicides (PPDB, 2017).

	Triasulfuron	Prosulfocarb
Chemical name (IUPAC)	1-[2-(2-chloroethoxy) phenylsulfonyl]-3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl) urea	*S*-benzyl dipropyl(thiocarbamate)
Chemical structure		
Water solubility (mg L^{-1})	815 (20°C, pH 7)	13.0 (20°C, pH 6.1)
log Kow	-0.59 (20°C, pH 7)	4.48 (20°C, pH 7)
DT_{50} (laboratory) (days)	59.1	12.4
DT_{50} (field) (days)	38.5	9.8
pKa	4.64 (at 25°C)	-
GUS index [a]	5.12	0.84

[a] GUS index, Groundwater Ubiquity Score is an experimentally calculated value that relates pesticide half-life (DT_{50}) and Koc (from laboratory data) (GUS= [log DT_{50}] [4 - log Koc]). It is an indicator of the potential risk of water pollution, which allow classifying pesticides into potential leachers (GUS > 2.8), non-leachers (GUS < 1.8) and transient leachers (1.8 < GUS < 2.8) (Gustafson, D.I., 1989. Groundwater ubiquity score: a simple method for assessing pesticide leachability. Environ. Toxicol. Chem. 8, 339–357).

Table S2

Validation parameters for determination of triasulfuron and prosulfocarb residues from unamended and green compost-amended soils by HPLC/MS analytical method.

Pesticide/ Soil sample	Recovery[a] %	RSD[a] n=3	r^{2b}	LOD[c] µg mL^{-1}	LOQ[c] µg mL^{-1}
Triasulfuron (Logran®)					
Soil	83.2±3.21	3.85	0.999	0.026	0.088
Soil + GC	80.4±4.71	4.96	0.998	0.018	0.059
Triasulfuron (AurosPlus®)					
Soil	82.9±2.24	3.26	0.999		
Soil + GC	80.2±5.27	6.15	0.999		
Prosulfocarb (Auros®)					
Soil	91.9±1.48	1.67	0.998	0.008	0.027
Soil + GC	90.4±4.13	5.62	0.999	0.005	0.017
Prosulfocarb (AurosPlus®)					
Soil	81.9±3.62	4.18	0.998		
Soil + GC	80.9±3.14	3.91	0.997		

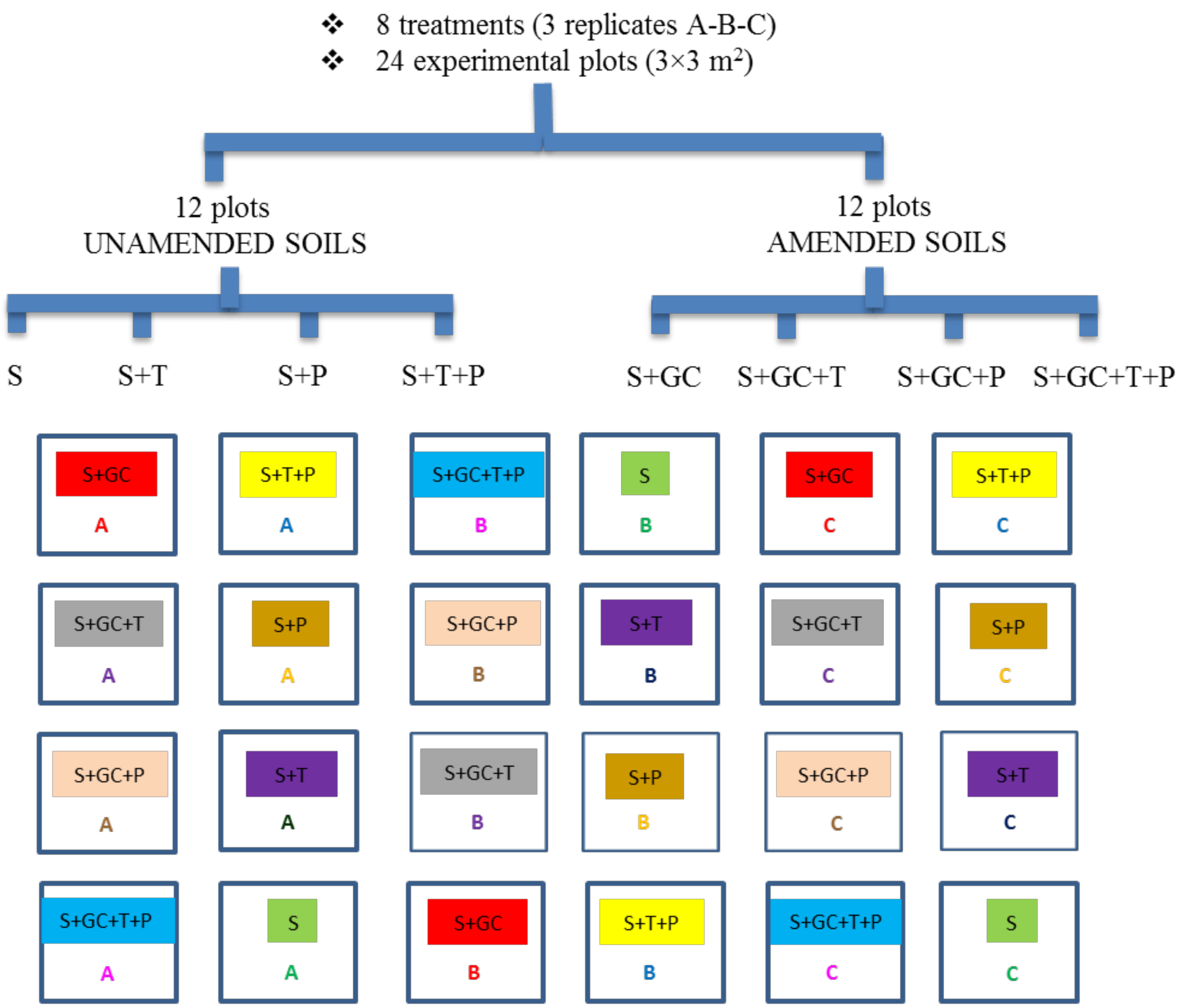
[a]The accuracy and precision of the proposed method were determined by recovery experiments corresponding to soil samples spiked with 76.9 µg kg^{-1} for triasulfuron or 3.46 mg kg^{-1} for prosulfocarb and the relative standard deviations (RSD) of the signals or peak areas obtained for each herbicide. [b]Lineal range 0.1-2.5 µg mL^{-1}; a matrix-matched calibration in soil extracts was used to offset the matrix effect and avoid any under/over estimation of herbicides. [c]LODs and LOQs were estimated as the analyte concentration with a signal-to-noise ratio of 3 and 10, respectively.

Table S3

Adsorption coefficients (K_d) of triasulfuron and prosulfocarb by unamended and green compost amended soil from experimental depths of soil profile.

Adsorption coefficient	Soil depth (cm)				
K_d	0-10	10-20	20-30	30-40	40-50
Triasulfuron (Logran®)	0.05±0.01[a]	0.13±0.11	0.23±0.06	0.22±0.01	0.18±0.03
	0.53±0.14[b]	-	-	-	-
Triasulfuron (Auros Plus®)	0.31±0.01[a]	0.30±0.04	0.28±0.04	0.32±0.04	0.29±0.01
	0.38±0.09[b]	-	-	-	-
Prosulfocarb (Auros®)	24.1±4.28[a]	18.6±4.53	11.6±0.22	7.40±0.66	3.13±0.77
	37.2±5.69[b]	-	-	-	-
Prosulfocarb (Auros Plus®)	21.6±5.55[a]	13.3±0.27	9.56±0.59	6.23±0.28	2.73±0.39
	30.7±7.62[b]	-	-	-	-

[a] Unamended soil, [b] Green compost amended soil



S+GC A	S+T+P A	S+GC+T+P B	S B	S+GC C	S+T+P C
S+GC+T A	S+P A	S+GC+P B	S+T B	S+GC+T C	S+P C
S+GC+P A	S+T A	S+GC+T B	S+P B	S+GC+P C	S+T C
S+GC+T+P A	S A	S+GC B	S+T+P B	S+GC+T+P C	S C

S=soil, S+GC=soil+green compost, T=triasulfuron, P=prosulfocarb

Figure S1

Experimental set-up

CAPÍTULO 2

Resumen

CAPÍTULO 2: Simultaneous application of two herbicides and green compost in a field experiment: Implications on soil microbial community

RESUMEN

La aplicación simultánea de herbicidas y enmiendas orgánicas es una práctica agrícola común que puede modificar el comportamiento de los herbicidas y afectar a la comunidad microbiana en los suelos. Hay poca información sobre los cambios en la comunidad microbiana del suelo como consecuencia de esta práctica agrícola en condiciones reales de campo.

El objetivo de este trabajo fue evaluar los efectos de dos herbicidas (triasulfuron y prosulfocarb) sobre la abundancia, actividad y estructura de la comunidad microbiana del suelo. Se llevó a cabo un experimento de campo aplicando dichos herbicidas en un suelo franco arcillo-arenoso en forma de formulaciones comerciales individuales, Logran® (triasulfuron) y Auros® (prosulfocarb), o conjunta, Auros Plus® (triasulfuron+prosulfocarb). Se establecieron parcelas experimentales de 9 m^2 en la finca experimental Muñovela del IRNASA-CSIC en un suelo sin enmendar y enmendado con un compost vegetal (GC) (120 t ha^{-1} ~ 11.6 t C ha^{-1}) procedente de la poda de árboles y plantas en parques y jardines. Se establecieron parcelas sin tratamiento de herbicidas y parcelas tratadas con las formulaciones comerciales Logran®, Auros® y Auros Plus® a las dosis de 4.5 kg i.a. ha^{-1} y 100 g i.a. ha^{-1} de prosulfocarb y triasulfuron, respectivamente. Se estudió la biomasa microbiana, la respiración, la actividad deshidrogenasa (DHA) y el perfil de ácidos grasos de fosfolípidos (PLFA) del suelo a diferentes tiempos durante el periodo de disipación de los herbicidas (100 días).

La aplicación del GC produjo un aumento en la biomasa de los suelos enmendados con respecto al suelo sin enmendar desde el principio del experimento. La aplicación de las formulaciones individuales de herbicidas no produjo diferencias significativas en los valores de biomasa de los suelos sin enmendar después de 30 días de tratamiento, aunque después de 100 días, aumentaron para todas las formulaciones de herbicidas. Sin embargo, los valores de biomasa disminuyeron en los suelos enmendados con la aplicación individual o combinada de triasulfuron en comparación con el control. Este efecto tóxico del herbicida triasulfuron sobre la biomasa microbiana fue temporal, ya que ésta se recuperó con la disipación del compuesto.

También la aplicación de GC aumentó los valores de respiración del suelo enmendado con respecto al suelo no enmendado pero en los suelos sin enmienda disminuyó en presencia de triasulfuron aplicado de manera individual o conjunta con prosulfocarb. Sin embargo, la aplicación individual de prosulfocarb no produjo una disminución significativa de la respiración. La enmienda GC inicialmente amortiguó el efecto del triasulfuron aplicado de manera individual, pero esta capacidad amortiguadora no resultó efectiva cuando se aplicó la formulación conjunta de los herbicidas.

La DHA del suelo no se vio afectada por la aplicación de GC o herbicidas después de 30 días desde su aplicación. Los valores de DHA disminuyeron con el tiempo en los suelos sin enmendar y tratados con los herbicidas de manera individual o conjunta, pero esta disminución no fue observada en los suelos enmendados. En presencia del herbicida triasulfuron aplicado de manera individual o conjunta los valores de DHA fueron constantes durante el periodo de estudio. Sin embargo, en el suelo tratado con prosulfocarb de manera individual la DHA aumentó de manera significativa después de 100 días.

La estructura microbiana cambió de manera clara durante la duración del experimento bajo las diferentes condiciones estudiadas. Después de 100 días desde la aplicación simultánea del triasulfuron y prosulfocarb hubo un aumento significativo de la población de bacterias Gram positivas y un descenso significativo en la población de bacterias Gram negativas y *Actinobacterias* en los suelos sin enmendar. El GC aplicado al suelo como enmienda mitigó el efecto de los herbicidas y sólo la abundancia relativa de *Actinobacterias* aumentó a los 100 días.

Según los resultados obtenidos, las comunidades microbianas en el suelo sin enmendar y enmendado con GC se comportaron de manera diferente con la aplicación de los herbicidas; además, la aplicación combinada de los compuestos alteró la actividad microbiana y su estructura en comparación con su aplicación individual o no aplicación. El uso del GC disminuyó el impacto del triasulfuron y del prosulfocarb en la biomasa y la actividad microbiana, y redujo las variaciones en la estructura microbiana del suelo.


Artículo

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Simultaneous application of two herbicides and green compost in a field experiment: Implications on soil microbial community

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ABSTRACT

The simultaneous use of herbicides and organic amendments is a common agricultural practice that may modify the behavior of the herbicides themselves and affect the microbial community in soils. There is little information about the changes in the soil microbial community by this agricultural practice under real field conditions. The aim of this work was to assess the effects on the soil microbial community (abundance, activity, and structure) of the following two herbicides triasulfuron (TSF) and prosulfocarb (PSC) applied as individual or combined formulations in an unamended soil (Soil) and in a soil amended with green compost (Soil + GC) at field scale. Herbicide dissipation, soil biomass, respiration, dehydrogenase activity (DHA), and the profile of phospholipid fatty acids (PLFA) were monitored for 100 days. Triasulfuron recorded a slower dissipation rate than PSC. The dissipation rate of TSF decreased in the GC-amended soils compared to the unamended ones. Furthermore, the Soil + GC recorded a higher soil biomass and respiration than the unamended ones. In the GC-amended soil, biomass values decreased with individual or combined TSF application compared to the Soil + GC control, while biomass values in the unamended soil increased with the application of combined herbicides after 100 days. In general, soil respiration values decreased with the application of herbicides in both the unamended and GC-amended soils. This negative effect was higher for the combined TSF + PSC application. DHA values decreased over time in the unamended soils treated with herbicides, but this decrease was not observed in the GC-amended soil. The microbial structure clearly changed throughout the experiment under the different conditions assayed. After 100 days of simultaneous TSF + PSC application, there was a significant increase in Gram-positive bacteria and a significant decrease in Gram-negative bacteria and *Actinobacteria* in the unamended soil. The GC-amended soil minimized the effects of TSF + PSC, and only the relative abundance of *Actinobacteria* increased at 100 days. The microbial community in the unamended and GC-amended soils behaved differently with herbicide application; however, the combined application of TSF and PSC altered soil microbial activity and structure compared to their individual application or non-application. The application of GC to soil buffered the impact of TSF and PSC on microbial biomass and activity, and reduced the shift in the soil microbial structure.

1. Introduction

The application of pesticides in modern agriculture is a widespread practice around the world designed to increase crop yields (Imfeld and Vuilleumier, 2012). However, the extensive use of these chemicals also releases pollutants into the environment (Herrero-Hernández et al., 2016; Pose-Juan et al., 2015b; Sánchez-González et al., 2013). Given the toxic nature of pesticides, considerable effort has been made to monitor, understand and minimize their environmental impact (Herrero-Hernández et al., 2015, 2016; Odukkathil and Vasudevan, 2013).

In this respect, the application of organic amendments to the soil could act as a barrier to avoid pesticide leaching, minimizing the spread of pollutants (Álvarez-Martín et al., 2016b; Marín-Benito et al., 2013, 2017). On the other hand, the use of organic amendments is a common practice in agriculture and in soil remediation processes for increasing the soil content of nutrients and organic carbon (OC) (Clemente et al., 2015; Medina et al., 2012). This agricultural practice improves soil properties and crop yield, and enhances soil microbial activity (López-Rayo et al., 2016; Medina et al., 2015; Zornoza et al., 2016). Organic amendments can inoculate new microorganisms in the soil or promote the growth of specific microorganisms that modify the soil's microbial

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activity and structure (Álvarez-Martín et al., 2016a; García-Delgado et al., 2015; Sun et al., 2017).

However, organic amendments may also modify the persistence and dissipation of pesticides in soils by increasing soil OC (Marín-Benito et al., 2012, 2013, 2014). In some cases, organic amendments have led to a decrease in the half-life of a pesticide, while in others there was an increase or even no effect at all (Álvarez-Martín et al., 2016a; Hussain et al., 2015; Marín-Benito et al., 2014). As the application of organic amendments affects the behavior of pesticides in the soil, they will be able to regulate the bioavailability and concentration of pesticides in the soil solution, conditioning their possible impact on soil microbial communities. Therefore, the soil microbial community's function and activity could be affected by the simultaneous application of pesticides and organic amendments (Hussain et al., 2009; Pose-Juan et al., 2015a).

Information about the effects pesticides have on soil microorganisms and assessing the toxicity of these compounds for soil microbial communities is increasing nowadays due to is a pre-requisite for improving pesticide regulation in the short term (Karpouzas et al., 2014). According to the reviews by Hussain et al. (2009) and Imfeld and Vuilleumier (2012), the presence of pesticides and their degradation products in the soil may inhibit, promote, or have no effects on microbial diversity and its functions. Therefore, considering the inconsistent results and the significance of microbial biomass, diversity and activity in many soil cycles and soil health, there is considerable scientific interest in determining the impact pesticides has on the soil microbial community (Martin-Laurent et al., 2010).

Moreover, there is little information about the soil microbial community's response and function when pesticides and organic residues are simultaneously applied (Pose-Juan et al., 2017, 2015a). Many of the published studies on soil microbial community's response to pesticides have been conducted at laboratory or greenhouse scale (Cycoń et al., 2012, 2013; Karpouzas et al., 2014; Pose-Juan et al., 2017, 2015a), while field-scale assays that replicate real conditions are scarce (Petric et al., 2016; Spyrou et al., 2009).

The largest share of pesticides involves herbicides, which play key roles in promoting crop yields. These compounds have also posed serious issues of environmental pollution (Huang et al., 2016), and soil bacteria are sensitive to some of them, such as sulfonylureas, affecting the universal biological processes in living systems (Patyka et al., 2016).

Two groups of herbicides widely used today are sulfonylureas and thiocarbamates. They have good selectivity, and are characterized by broad-spectrum weed control for many cereal crops, such as rice, wheat or maize, soybean and sugar beet or vegetables (e.g., carrots, peas, and potatoes) (Sofo et al., 2012). Triasulfuron is a sulfonylurea that inhibits the behavior of acetolactate synthase, and it is responsible for the biosynthesis in plants and bacteria of three branched-chain amino acids (leucine, isoleucine, and valine). Since the enzyme is absent in humans and animals, it is a safe choice to apply sulfonylureas in the field (Wang et al., 2010). Triasulfuron is a weak acid which presents a high solubility in water and low hydrophobicity. In field dissipation studies, TSF exhibited an elevated mobility and moderate persistence in soils (PPDB, 2017). The time required for the concentration to decline to half of the initial value (DT_{50}) ranged between 15.9 and 65.4 days (EFSA, 2015). The adsorption of TSF by soils influences its biodegradation and bioavailability (Said-Pullicino et al., 2004). Its transformation to metabolites is due to microbial degradation and chemical hydrolysis (Pose-Juan et al., 2017; Singh and Kulshrestha, 2006). Soil bacterial communities or activities could be affected by this herbicide (Pose-Juan et al., 2017; Wang et al., 2010). Nevertheless, little is currently known about the impact TSF and other sulfonylurea herbicides have on soil microbes (Karpouzas et al., 2014).

Thiocarbamate herbicides inhibit the elongase enzyme, hence the main effect of these herbicides is the inhibition of the synthesis of very-long-chain fatty acids, while also affecting meristematic tissues. Among these herbicides, and as a secondary effect, PSC has previously been reported to inhibit shikimate synthesis, leading to a decrease in flavonoid content and a variation in amino acid composition and content. The changes can be interpreted as secondary effects, probably related to the stress caused by the primary effects of PSC (Hjorth et al., 2006). Prosulfocarb has low solubility in water and high hydrophobic character. This herbicide presents high adsorption, is slightly mobile and non-persistent in soils (PPDB, 2017). Under field conditions, prosulfocarb DT_{50} values ranged between 6.5 and 13.0 days (EFSA, 2007). The dissipation of PSC is due mainly to microbial degradation (Gennari et al., 2002). The high adsorption of PSC by soil organic matter fractions could lead to a decrease in leaching (Nègre et al., 2006).

The physicochemical behavior of TSF and PSC herbicides, including their dissipation, mobility and persistence in a field experiment in an unamended soil and one amended with green compost (GC), has been evaluated in a previous study (Marín-Benito et al., 2018). Herbicide concentrations were determined at various times in the surface soil and at different depths up to 50 cm to evaluate the effect of the increased OC in the amended soil and the influence on the dissipation and mobility of individual Logran® and Auros®, or the combined commercial formulation Auros Plus® of both compounds.

The present work supports a simultaneous study designed to evaluate the effect of herbicides applied on microbial communities and their evolution over the dissipation process. To our knowledge, there are no studies on the impact of PSC on soil microbial communities, and little is known about the impact of TSF on soil microbes under real field conditions (Karpouzas et al., 2014).

Accordingly, the aim of this work was to determine the possible modifications of soil microbial communities due to the application of the herbicides TSF and PSC on an unamended and a GC-amended soil. A field experiment was set up, and the effects of the individual or combined commercial formulations of the herbicides were studied on the following: (i) the soil microbial biomass, respiration, and dehydrogenase activity, as parameters indicating the abundance, overall activity and function of microbial communities, and (ii) the profile of phospholipid fatty acids (PLFAs) extracted from the soil, as indicator of the soil microbial structure. Changes were evaluated at various times during the dissipation of herbicides in the soil surface.

2. Materials and methods

2.1. Herbicides

This study used the commercial formulations of triasulfuron (TSF) (Logran® 20% p/p), prosulfocarb (PSC) (Auros® 80% p/v), and triasulfuron + prosulfocarb (TSF + PSC) (Auros Plus®) (Syngenta Agro S.A., Madrid, Spain). Analytical standards of both compounds were purchased from PESTANAL® (purity > 98.9%) (Sigma Aldrich Química S.A., Madrid, Spain). The chemical name, structure and characteristics of these compounds are included in Table S1 (in Supplementary material) (PPDB, 2017).

2.2. Green compost

A composted organic residue of vegetal origin from the pruning of plants and trees in parks and gardens in the city of Salamanca (Spain) has been used. It was supplied by the city council. The physicochemical characteristics of this green compost (GC) on a dry weight basis are as follows: pH 7.33, determined in a GC/water suspension (1:2); OC content 9.80%, determined by the modified Walkley-Black method; dissolved organic carbon (DOC) 0.353%, determined in a suspension of GC in deionized water (1:2) after shaking (24 h), centrifuging (20 min at 10,000 rpm) and filtering, using a Shimadzu 5050 (Shimadzu, Columbia, MD, USA) organic carbon analyzer; total N, 1.04% determined by the Kjeldahl method. The C/N rate was 9.42, and the ash percentage determined by weight difference after ignition at 540 °C for

24 h was 74.5%.

2.3. Experimental set-up

A field experiment was conducted with an agricultural sandy clay loam soil (Typic Haploxerept) (Soil Survey Staff, 2010) on the Muñovela experimental farm belonging to the Institute of Natural Resources and Agrobiology of Salamanca (IRNASA-CSIC), Spain (40°55′56″ N latitude and 5°52′53″W longitude). A detailed description of the experimental layout of randomized complete blocks of unamended soil (Soil) (12 plots) and soil amended with GC (Soil + GC) at the rate of 120 t ha^{-1} on a dry weight basis (~11.6 t C ha^{-1}) (12 plots) and of the weather conditions is included in Supplementary material and in Marín-Benito et al. (2018).

Water herbicide suspensions were applied manually using a backpack sprayer (volume of 10 L) seven days after the soil was amended. The doses applied to the plots were in the ranges of the recommended application doses for both herbicides (4.5 kg a.i. ha^{-1} as Auros® 80% (PSC), and 100 g a.i. ha^{-1} as Logran® 20% (TSF)). Similar doses of both compounds were applied jointly as Auros Plus® (TSF + PSC). The combination of soil management, unamended (Soil) and amended with GC (Soil + GC), and herbicides application (TSF, PSC or TSF + PSC) denoted the treatments applied. A check was made prior to the application of the herbicides to ensure that no amounts of these compounds were detectable in the soil samples. This was as expected, because the plots had no record of TSF and PSC application in the previous five years.

2.4. Soil sampling, sample processing, and herbicide extraction and analysis

Surface soil samples from 0 to 10 cm were collected on the first day after herbicide application (0 days), and at 30 and 100 days after treatment to determine soil biomass, dehydrogenase activity (DHA), respiration, and phospholipid fatty acids (PLFAs) in all the treatments. Soil samples were also collected at the same times to determine herbicide dissipation. Five sub-samples were taken in each plot, mixing them before they were transferred to polypropylene bottles. All the samples were transported to the laboratory in portable refrigerators. Soil samples were divided into sub-samples to determine soil biomass, respiration, DHA and herbicide dissipation, which were analyzed immediately. To determine PLFAs, the samples were frozen at −80 °C and lyophilized prior to extraction and analysis. Soil characteristics were determined according to Marín-Benito et al. (2018), and are included in Supplementary material (Table S2).

Duplicate subsamples of moist soil (6 g) were extracted from each plot with methanol (12 mL) to determine herbicide residues. The samples were sonicated for 1 h, shaken at 20 °C for 24 h, and then centrifuged at 5045*g* for 15 min. The extracts were filtered to remove particles > 0.45 µm in a GHP Acrodisc filter (Waters Corporation) and evaporated until dryness at 25 °C under a nitrogen stream using an EVA-EC2-L evaporator (VLM GmbH, Bielefeld, Germany). The residue was dissolved in 0.5 mL of methanol + formic acid (1%), and transferred to a HPLC glass vial for analysis. The analysis of TSF and PSC was performed by HPLC attached to a ZQ mass spectrometer detector (MS) (Waters Assoc., Milford, MA, USA). A detailed description of the analytical method is included in Supplementary material.

2.5. Soil biochemical properties and PLFA analysis

Microbial biomass-N was extracted using the chloroform fumigation-extraction technique (Vance et al., 1987). Cytoplasm content was extracted with K_2SO_4, and the ammonium present was determined by colorimetry with a segmented flow autoanalyzer. A conversion factor into biomass-C of 21 was used (García-Izquierdo, 2003).

Soil respiration was determined by measuring the depletion of pressure produced by O_2 consumption by the microorganisms in 50 g of fresh soil over four days using OxiTop Control BM6 containers with an OxiTop Control OC 110 measurement system (WTW, Weilheim, Germany). The CO_2 produced by the metabolism of soil microorganisms was trapped in 10 mL of NaOH 1 M.

Soil DHA is a measure of overall microbial activity. This parameter was determined by the method of Tabatabai modified by Singh et al. (2002). Briefly, six grams of fresh soil were mixed with 60 mg of calcium carbonate and 1 mL 3% 2,3,5-triphenyltetrazolium chloride and 2.5 mL of ultrapure water. The reaction mixture was incubated at 37 °C for 24 h in the dark. At the end of incubation, the 1,3,5-triphenylformazan (TPF) was extracted with 7 mL of methanol, centrifuged (3000 rpm, 10 min) and extracted two times again. The three fractions were mixed and diluted to 25 mL with methanol. The absorbance of the supernatant was measured in a spectrophotometer at 485 nm. The results were expressed as µg TPF g^{-1} dry soil.

The microbial community composition of the soil samples was determined using PLFA analysis, as described in Pose-Juan et al. (2015a). Briefly, samples were freeze-dried, and 2 g of dry material was used for lipid extraction. Lipids were extracted with a one-phase chloroform-methanol-phosphate buffer solvent. Phospholipids were separated from non-polar lipids and converted to fatty acid methyl esters before analysis. Quantification was performed using an Agilent 7890 gas chromatograph (Agilent Technologies, Wilmington, DE, USA) equipped with a 25-m Ultra 2 (5% phenyl)-methylpolysiloxane column (J&W Scientific, Folsom, CA, USA) and a flame ionization detector. PLFAs were identified using bacterial fatty acid standards and software from the Microbial Identification System (Microbial ID, Inc., Newark, DE, USA).

2.6. Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) being the main factors soil treatments and sampling times. Duncan or Games–Howell post hoc test (according to Levene variance homogeneity test) at $p < 0.05$ was used to determine significant differences between means and evaluate the effects of the different soil treatments at the same sampling time and the sampling times within the same soil treatment on the microbial biomass, respiration, DHA and PLFAs of soils. Pearson correlation coefficients between the remaining percentages of herbicides, soil OC, and microbial structure and activity were determined to elucidate how variables are related to each other. ANOVA and correlation analyses were carried out using the IBM SPSS Statistics v24 software package. Finally, principal component analysis (PCA) was performed, with PAST v3.15 software (Hammer et al., 2001), to determine the most meaningful variables and the global impact of the herbicides and GC on soil microbial communities.

3. Results and discussion

3.1. Dissipation of herbicides

Table 1 shows the remaining percentages of TSF and PSC at 30 and 100 days after the application of individual or combined herbicide

Table 1
Remaining percentages of triasulfuron and prosulfocarb in unamended soil and green compost amended soil at 30 and 100 days after application of individual or combined formulations of herbicides.

Herbicide	Soil		Soil + Green Compost	
(Formulation)	30 days	100 days	30 days	100 days
Triasulfuron (Logran®)	29 ± 7	2.2 ± 0.2	62 ± 15	7.9 ± 3.8
Prosulfocarb (Auros®)	25 ± 17	0.4 ± 0.1	26 ± 10	1.4 ± 0.6
Triasulfuron (Auros Plus®)	51 ± 18	2.4 ± 2.2	41 ± 7.1	9.4 ± 3.2
Prosulfocarb (Auros Plus®)	37 ± 17	0.7 ± 0.4	20 ± 5.1	2.1 ± 0.7

formulations. The concentrations measured at the beginning of the dissipation study ranged between 0.073 and 0.211 mg TSF kg^{-1} dry soil and 4.56–5.22 mg PSC kg^{-1} dry soil, respectively, in different plots. At 30 days after herbicide application, the remaining percentages of TSF and PSC were between 29% and 62% and between 20% and 37% of the initial concentrations, respectively, for the different conditions studied. At 100 days after herbicide application, there was almost no further dissipation of either TSF or PSC, with the remaining percentages of TSF and PSC being between 2.2% and 9.4% and 0.4% and 2.1%, respectively. PSC therefore recorded a higher dissipation rate than TSF under the different conditions assayed.

The GC-amended soil recorded slower TSF dissipation than the unamended soil. However, the dissipation of PSC was not affected by GC application. Pose-Juan et al. (2017) described the decrease of TSF dissipation in the soil amended with GC compared to the unamended soil at laboratory scale. The decrease in TSF dissipation in Soil + GC could be due to a decrease in microbial degradation caused by herbicide adsorption by soil organic matter. Adsorption reduces pesticide solubility in a soil solution and its bioavailability to microbial degradation, increasing the presence of pesticides in the top soil (Herrero-Hernández et al., 2015; Marín-Benito et al., 2013). In contrast, the fact there were no differences for PSC dissipation between the GC-amended and unamended soils could be due to two factors: firstly, this compound's high hydrophobicity (low water solubility, Table S1) may lead to high adsorption in both the GC-amended and unamended soils; secondly, the possible losses by volatilization of the parent compound (Braun et al., 2017). A detailed description of the dissipation kinetics and mechanisms of TSF and PSC in the GC-amended and unamended soils has been reported in Marín-Benito et al. (2018).

3.2. Soil microbial biomass and activity

3.2.1. Soil microbial biomass

Fig. 1 shows the evolution of C-microbial biomass in the unamended and GC-amended soils either untreated or treated with herbicides. No significant differences were detected in the microbial biomass in the control soil (Soil) between sampling times with the mass remaining constant during the assay. At short and medium time (0–30 days), the individual application of the herbicides TSF and PSC in the unamended soil did not modify the microbial biomass compared to the control treatment. However, 100 days after herbicide application the biomass increased in the presence of TSF and/or PSC in the unamended soil, with the combination of TSF + PSC producing a significant increase ($p < 0.05$) in microbial biomass compared to the control soil and the soil treated individually with TSF or PSC.

Toxic effects of TFS and other sulfonylurea herbicides were reported at laboratory scale for field or higher doses after 30 days of incubation (Sofo et al., 2012). In this work, a certain toxicity of TSF was observed in Soil + TSF after 30 days. Biomass decreased (Fig. 1) compared to initial time, although at the end of the assay the microbial biomass in Soil + TSF and Soil + TSF + PSC increased to values similar to those at the beginning of the assay. This means the toxic effects of TSF were limited in time, and the microbial biomass recovered with TSF dissipation. Lupwayi et al. (2004) have not reported any significant effects of a field dose of TSF on C-microbial biomass at field scale. Similarly, in a field experiment, butachlor applied at the recommended dose had not significant effect on C-microbial biomass (Singh et al., 2016). On the contrary, the two-year application of the herbicide imazethapyr to soybean fields increased the C-microbial biomass indicating that the herbicide itself might provide a carbon source for soil microorganisms (Zhang et al., 2010).

The application of GC to soil enhanced the microbial biomass over that of the unamended soil from the beginning of the assay, with the detection of significant differences between sampling times (Fig. 1). The increase in microbial biomass after GC application has already been reported in herbicide dissipation studies at laboratory scale (Pose-Juan et al., 2015a,c, 2017) or for other organic amendments at field scale (Singh et al., 2016). Neither did the individual application of herbicides modify the increased microbial biomass observed in the Soil + GC over the short and medium term (0–30 days) as in the unamended soil. Soil + GC buffered the effects of these herbicides, and significant differences were observed solely in the evolution of each treatment at 100 days after herbicide application. At this time in contrast to the unamended soil, TSF applied individually (Soil + GC + TSF) or in combination with PSC (Soil + GC + TSF + PSC) led to a decrease ($p < 0.05$) in microbial biomass with respect to Soil + GC especially in

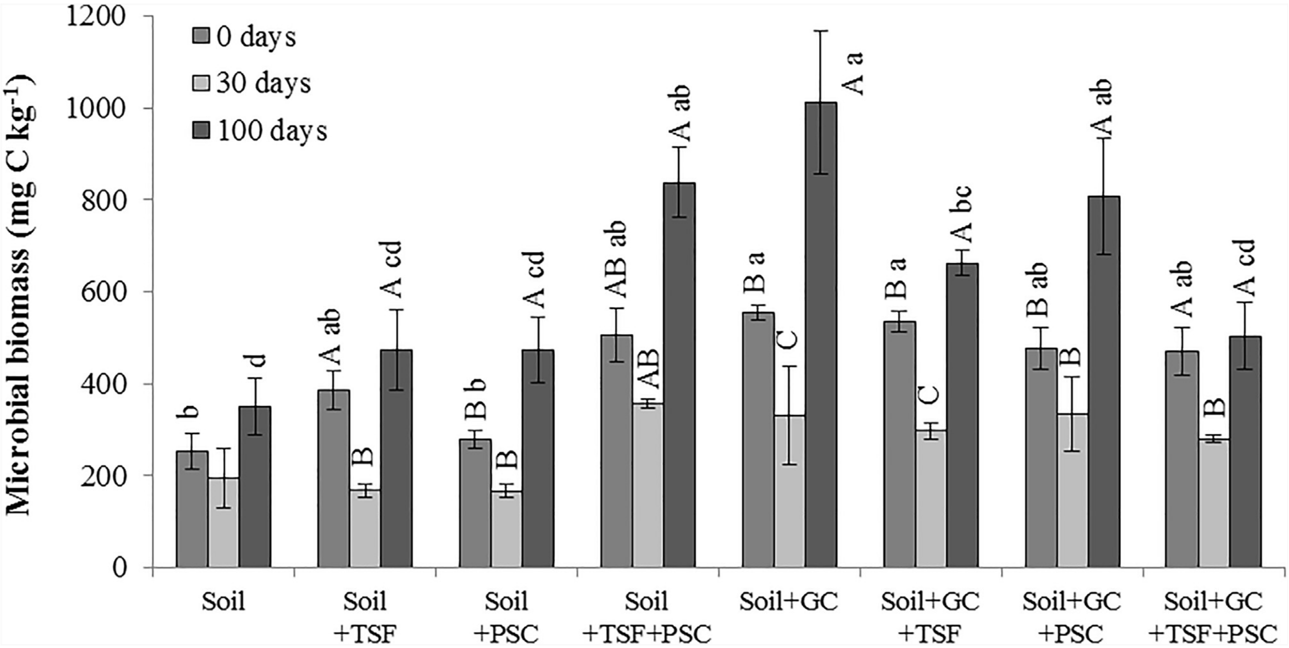

Fig. 1. Microbial biomass for unamended soil (Soil) and soil amended with green compost (Soil + GC) in absence or presence of triasulfuron (TSF) and/or prosulfocarb (PSC). Data present the mean ± standard deviation of three replicated plots. Different lowercase and uppercase letters indicate significant differences between treatments at the same sampling time and between sampling times within the same treatment (Duncan post hoc test; $p \le 0.05$), respectively. Lack of letters indicates no significant differences.

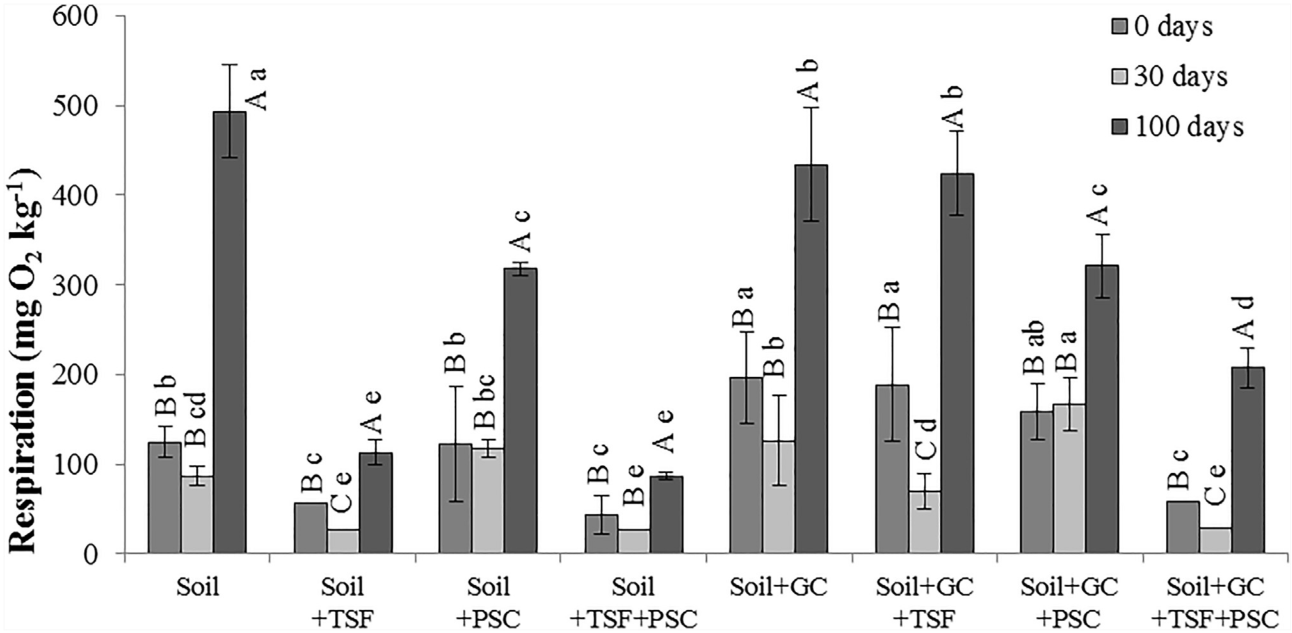

Fig. 2. Soil respiration for unamended soil (Soil) and soil amended with green compost (Soil + GC) in absence or presence of triasulfuron (TSF) and/or prosulfocarb (PSC). Data present the mean ± standard deviation of three replicated plots. Different lowercase and uppercase letters indicate significant differences between treatments at the same sampling time and between sampling times within the same treatment (Duncan post hoc test; $p \leq 0.05$), respectively.

combination with PSC. With respect to initial values, microbial biomass in Soil + GC treatments tends to increase at the end of the assay, except in Soil + GC + TSF + PSC, where the microbial biomass did not record any significant differences between the initial and final sampling times, which confirms the negative effect of the combined application of both herbicides, TSF and PSC. These effects could be due to the higher amounts of herbicides remaining at 100 days in Soil + GC (Table 1) compared to unamended soil, which may have a toxic effect on the microbial biomass. Pose-Juan et al. (2017) have reported an increase of microbial biomass at a low dose of TSF in a soil amended with GC during the incubation period. The general reduction in microbial biomass at 30 days in all the treatments could be due to external factors such as weather conditions or low moisture in the surface soil (Marín-Benito et al., 2018).

3.2.2. Soil respiration

Fig. 2 presents the soil respiration results, expressed as mg of O_2 consumed per kg of dry soil. Soil respiration was very sensitive to treatments and sampling times. Respiration of the unamended soil decreased at initial time, and at 30 days after TSF was applied individually or combined with PSC (Soil + TSF and Soil + TSF + PSC). However the individual application of PSC (Soil + PSC) did not record any significant decreasing effect ($p > 0.05$) regarding the Soil control. Sofo et al. (2012) have also described the inhibition of soil respiration over 30 days of TSF application, although other sulfonylureas either promoted or had no effect on soil respiration at 30 days of incubation. Finally, at 100 days after herbicide application, respiration in soils Soil + TSF, Soil + PSC, and Soil + TSF + PSC was higher ($p < 0.05$) than at 0 or 30 days. This is in agreement with the increase in microbial biomass (Fig. 1), but soil respiration in presence of herbicides was reduced compared to the Soil control ($p < 0.05$) in spite of the remaining amounts of herbicide at this time were low. Similarly, the application of the herbicide fomesafen at the field dose resulted in significantly lower basal respiration rates during the first 15 days, whereas it was significantly lower at all incubation times in soil treated with higher doses of herbicide (Wu et al., 2014).

The addition of GC enhanced soil respiration with respect to the unamended soil ($p < 0.05$), and initially buffered the effect of the individual application of TSF (Soil + GC + TSF), which did not modify the respiration with respect to the Soil + GC control. However, the buffer capacity of GC was ineffective for the combined application of TSF and PSC (Soil + GC + TSF + PSC), which reduced soil respiration to the values of the unamended soil (Soil + TSF and Soil + TSF + PSC). At 30 days after herbicide application, respiration also tended to decrease in the soils treated with TSF (Soil + GC + TSF), but soils treated with PSC recorded significant increase compared with the Soil + GC control. Prosulfocarb increased soil respiration as it was reported in other laboratory experiments in GC amended soil in the presence of TSF (Pose-Juan et al., 2017) or high doses of mesotrione (Pose-Juan et al., 2015c). An increase of respiration was observed at 100 days after herbicide application in GC amended soils ($p < 0.05$), but soil respiration was reduced in Soil + GC + PSC and Soil + GC + TSF + PSC compared to the Soil + GC control ($p < 0.05$) as it was observed in unamended soils.

3.2.3. Soil dehydrogenase activity (DHA)

Fig. 3 presents the DHA values for the unamended and GC-amended soils either untreated or treated with herbicides. The DHA values were not affected by GC or herbicide application at 0 and 30 days of assay. At 100 days, there were no significant differences between the control soil (Soil) and the soil treated with herbicides (Soil + TSF, Soil + PSC and Soil + TSF + PSC). The DHA in Soil was constant over the 100 days of assay; in contrast, the DHA values decreased over time for treatments Soil + TSF, Soil + PSC, Soil + TSF + PSC ($p < 0.05$), reflecting the impact of herbicides or their metabolites on microbial activity. However, the herbicide napropamide applied at field rates had a negative impact on DHA at the beginning of the experiment (Cycoń et al., 2013).

DHA values also decreased in Soil + GC over time. A similar trend has been observed in a previous study using soil amended with GC at laboratory scale (Pose-Juan et al., 2015a). The soil microbiota retained their functional activity despite the sampling time and the individual or combined application of herbicides in Soil + GC. The DHA values increased significantly at 100 days in Soil + GC + PSC ($p < 0.05$). However, the DHA values were constant in Soil + GC + TSF and Soil + GC + TSF + PSC treatments over the assay period, being similar or higher than the DHA values in the Soil + GC control. The application of GC therefore buffered the negative effects of TSF and PSC in soil DHA over time. A similar conclusion has been reported for the herbicide

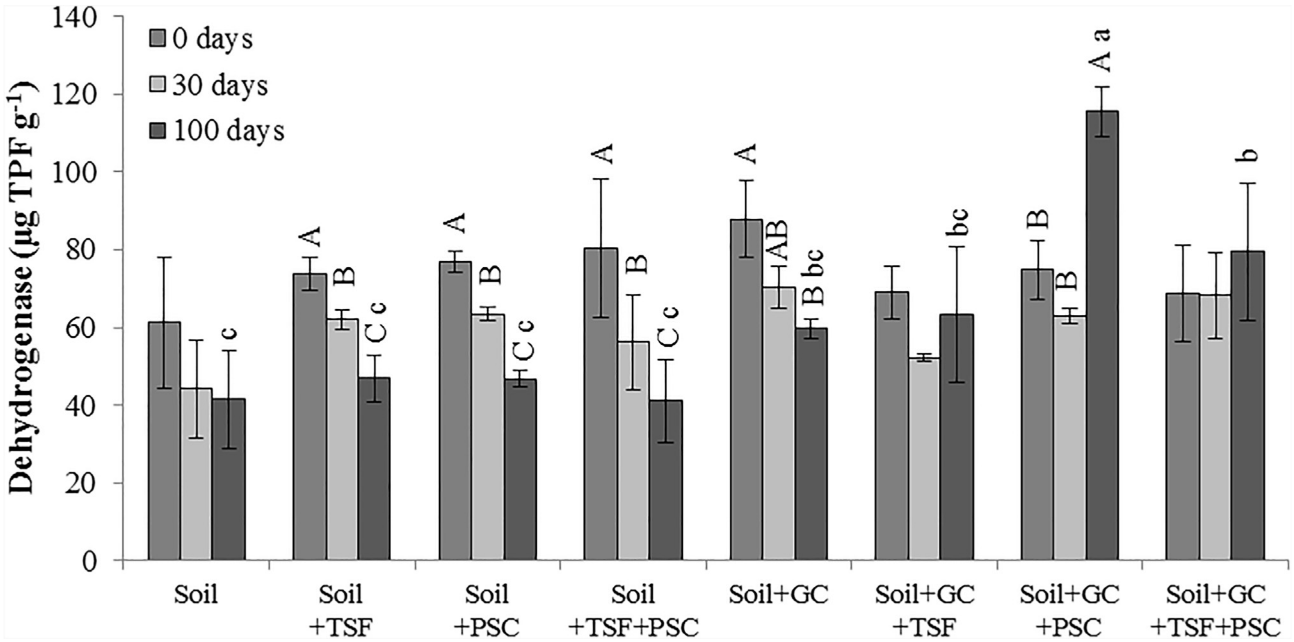

Fig. 3. Dehydrogenase activity for unamended soil (Soil) and soil amended with green compost (Soil + GC) in absence or presence of triasulfuron (TSF) and/or prosulfocarb (PSC). Data present the mean ± standard deviation of three replicated plots. Different lowercase and uppercase letters indicate significant differences between treatments at the same sampling time and between sampling times within the same treatment (Duncan post hoc test; $p \leq 0.05$), respectively. Lack of letters indicates no significant differences.

oxyfluorfen, which recorded a lower inhibition of enzymatic activities, including DHA, when organic wastes were added to soils (Gómez et al., 2014).

3.3. Phospholipid fatty acids profile analysis

Fig. 4 shows the relative abundance of PLFAs that specifically diagnose Gram-negative and Gram-positive bacteria, *Actinobacteria,* and fungi at seven days before herbicide application, and at 0, 30 and 100 days after herbicide application in the unamended and GC-amended soils.

Previously to application of herbicides the relative abundance of PLFAs in the plots of unamended soil and in the plots of GC-amended soil was analyzed. The results revealed no significant differences in the relative abundance of Gram-positive and Gram-negative bacteria, *Actinobacteria,* and fungi between plots of unamended soil or between plots of amended soil. Consequently a homogeneous microbial structure was recorded for Soil plots and for Soil + GC plots respectively. However, the comparison of microbial structure of Soil plots with Soil + GC plots indicated the application of GC to soil decreased ($p < 0.05$) the abundance of fungi with respect to the unamended soil (Fig. 4). This shift in the soil microbial structure immediately after GC addition (and previous herbicide application) was due to the input of new microorganisms from the compost's inherent microbial population (García-Delgado et al., 2015).

At 0 days of herbicide application, no significant differences were observed between Soil or Soil + GC controls and these soils treated with TSF, PSC or TSF + PSC. This means there was no modification of the microbial structure immediately after individual (TSF or PSC) or combined (TSF + PSC) herbicide application. Neither did a previous work on TSF dissipation at laboratory scale report any significant changes in unamended and GC-amended soils even at a very high concentration (50 mg kg^{-1}) immediately after herbicide application (Pose-Juan et al., 2017). However, other herbicides such as napropamide, acetochlor or MCPA significantly shifted the microbial community structure at the beginning of the incubation (Bai et al., 2013; Cycoń et al., 2013; Saleh et al., 2016).

At 30 days after herbicide application in the unamended soil, a significantly higher abundance of fungi was detected in Soil + TSF + PSC compared to the Soil control or Soil + TSF and Soil + PSC ($p < 0.05$). This higher abundance of fungi was offset by a decrease in Gram-positive bacteria and no changes were detected in Gram-negative bacteria or *Actinobacteria*. By contrast in Soil + GC, the combined application of TSF + PSC increased the abundance of Gram-positive bacteria ($p < 0.05$) and the application of individual TSF led to a decrease in fungi ($p < 0.05$). The relative abundance of *Actinobacteria* and Gram-positive bacteria were higher in Soil + GC + TSF and Soil + GC + TSF + PSC than in their respective treatments in the unamended soil ($p < 0.05$), but there was a lower abundance of fungi in Soil + GC + TSF + PSC than in Soil + TSF + PSC ($p < 0.05$).

At 100 days, there were significant differences ($p < 0.05$) between the treatments for Gram-negative and Gram-positive bacteria and *Actinobacteria*, but not for fungi. The lack of significant differences for fungi could be because fungi belong to the group of microorganisms that after an initial sensitive response to the presence of pesticides in the soil rapidly establish a normal metabolism (Kalia and Gosal, 2011). Overall, herbicides do not have a negative impact on the soil fungal population when applied at the recommended doses (Kalia and Gosal, 2011). At this time (100 days), the simultaneous application of TSF + PSC in the unamended soil led to a significant increase in the relative abundance of Gram-positive bacteria and a decrease in Gram-negative bacteria ($p < 0.05$) compared to Soil and Soil + TSF or Soil + PSC. This means that only the combined application of both herbicides modified bacterial diversity, and no significant shift was detected in the soils treated individually with TSF or PSC. This effect was buffered in the GC-amended soil, and no significant differences were found in the relative abundance of Gram-positive and Gram-negative bacteria. In contrast, the relative abundance of *Actinobacteria* increased ($p < 0.05$) in Soil + GC treated with individual or combined herbicides. Cycoń et al. (2012) found an increased amount of bacterial and fungal PLFAs in a soil after application of teflubenzuron at the end of the incubation time possibly due to the utilization of insecticide by soil microorganisms.

The ratio Gram-positive/Gram-negative bacteria (Fig. 5) decreased significantly over time for all the treatments ($p < 0.05$), except for

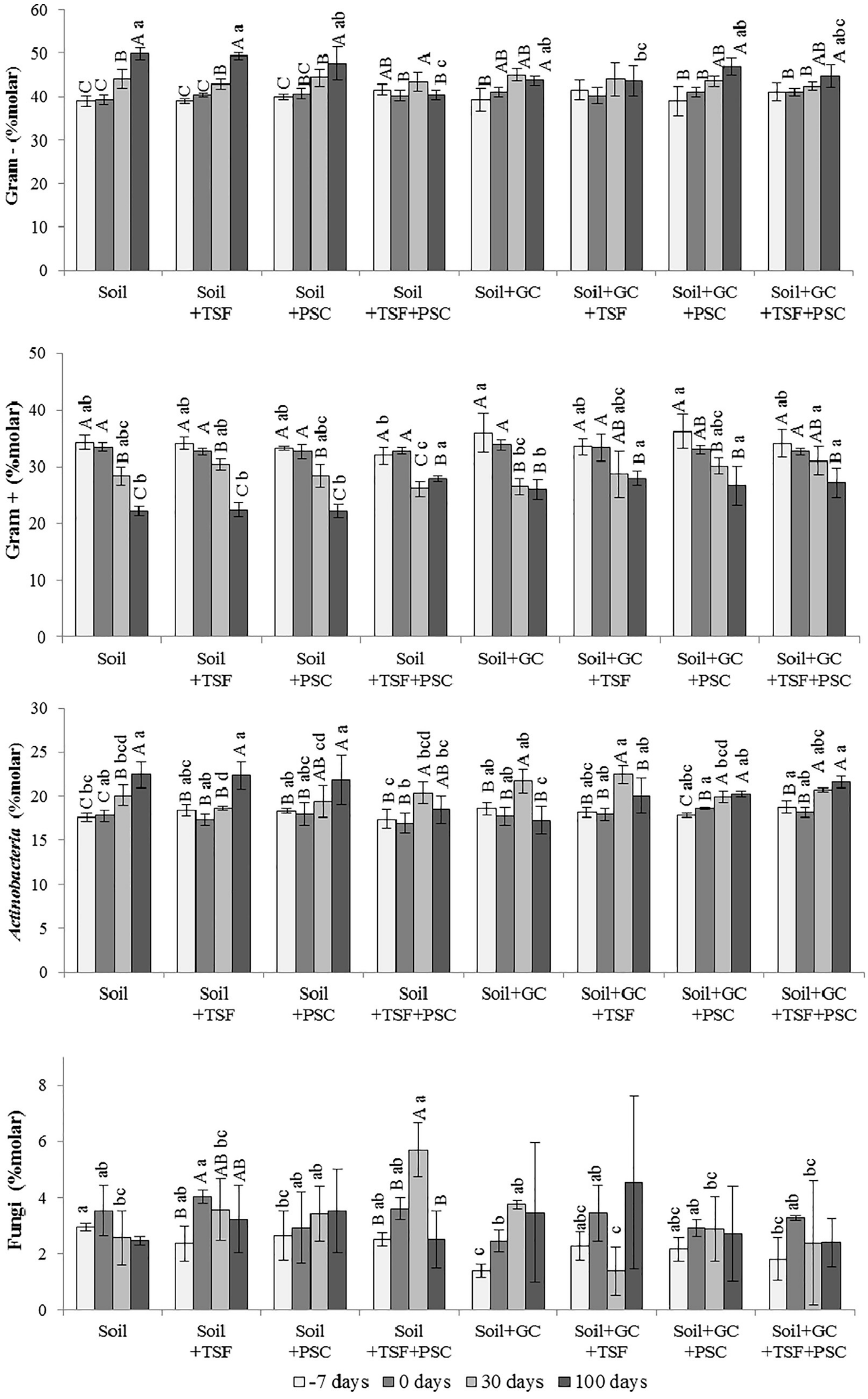

Fig. 4. Relative abundance (% mol) of PLFAs specifically diagnostic of Gram-negative and Gram-positive bacteria, *Actinobacteria* and fungi in the unamended soil (Soil) and soil amended with green compost (Soil + GC) before (-7 days) and after (0, 30 and 100 days) triasulfuron (TSF) and/or prosulfocarb (PSC) application. Data present the mean ± standard deviation of three replicated plots. Different lowercase and uppercase letters indicate significant differences between treatments at the same sampling time and between sampling times within the same treatment (Duncan post hoc test; $p \leq 0.05$), respectively. Lack of letters indicates no significant differences.

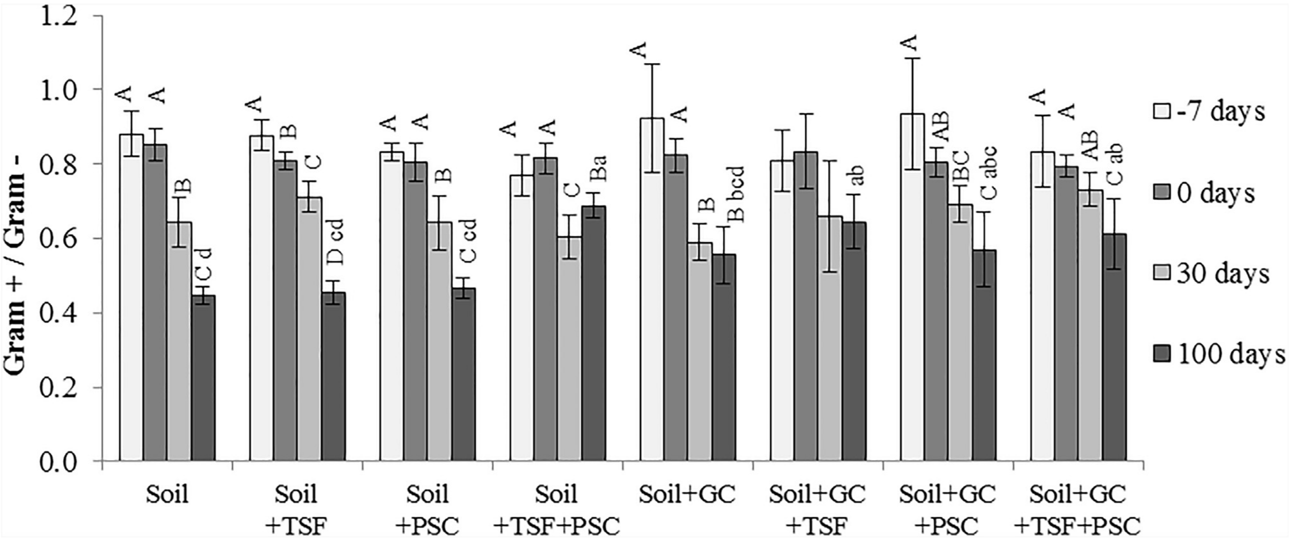

Fig. 5. Ratio Gram-positive/Gram-negative bacteria in unamended soil (Soil) and soil amended with green compost (Soil + GC) in absence or presence of triasulfuron (TSF) and prosulfocarb (PSC). Data present the mean ± standard deviation of three replicated plots. Different lowercase and uppercase letters indicate significant differences between treatments at the same sampling time and between sampling times within the same treatment (Duncan post hoc test; $p \leq 0.05$), respectively.

Soil + GC + TSF, which did not record any significant differences. At 100 days after herbicide application, the ratio Gram-positive/Gram-negative bacteria was significantly higher ($p < 0.05$) in Soil + TSF + PSC than in Soil and Soil + TSF or Soil + PSC. However, no significant differences were found between treatments in the GC-amended soils. Therefore, GC was able to buffer the microbial shift produced by the combined application of TSF + PSC. Pose-Juan et al. (2015a) have described a shift in the bacteria ratio towards Gram-negative bacteria in the Soil + GC soil over 98 days of incubation. They have reported that both the overall structure of active microbial communities and the relative abundance of certain groups of microorganisms clearly change according to the type of amendment and the time of incubation, but remain unaffected by the application of the herbicide mesotrione.

A statistical analysis of each microbial group's trend over time in untreated soils and those treated with herbicides showed a clear shift in microbial diversity. During the assay, there was an increase in the relative abundance of Gram-negative bacteria and *Actinobacteria* in the unamended and GC-amended soils, whereas the relative abundance of Gram-positive bacteria decreased, and the percentage of fungi remained almost the same (Figs. 4 and 5). It has been observed that Gram-negative bacteria can multiply rapidly in the presence of additional carbon sources (Cycoń et al., 2012) and fungi are more sensitive to chemical stresses (Wu et al., 2014). Similarly, Zhang et al. (2010) reported an increase in Gram-negative bacteria by the application of the herbicide imazethapyr for two years in a soybean field suggesting the herbicide may act as a carbon source. However, in a field study, application of nicosulfuron at agronomical rate did not significantly affect the abundance of fungi and bacteria, and did not induce large alterations in the soil microbial structure (Karpouzas et al., 2014).

3.4. Global impact of herbicides and green compost on microbial communities

Table 2 shows the Pearson correlation coefficients between the percentage of remaining herbicides, soil OC, and microbial structure and activity, and Fig. 6 presents the principal component analysis (PCA) of these variables. The combination of both analyses shows how some variables are related to each other. The relative percentage of Gram-positive bacteria negatively correlates with the relative percentage of Gram-negative bacteria and *Actinobacteria* (Table 2). This is clearly shown in the loading factors of PCA (Fig. 6), where Gram-positive bacteria and Gram-negative bacteria and *Actinobacteria* are opposite and strongly related to PC1. Significant positive correlations are found between microbial biomass and soil respiration, microbial biomass and OC and DHA and OC, suggesting that microbial biomass and activity and soil OC are interrelated. This has been confirmed with the PCA, where all these variables are positively related to PC2 (Fig. 6). Organic amendments have a positive effect on soil microbial biomass and DHA because organic amendments are the carriers of new microbial populations, and the input of new available OC stimulates microbial activity (Álvarez-Martín et al., 2016a; García-Delgado et al., 2015; Pose-Juan et al., 2015c).

The scores for each treatment and sampling time on the PCA (Fig. 6) show the different evolution of each treatment during the field assay. At 0 days after herbicide application, all the treatments were in the negative zone of PC1, positively related to Gram-positive bacteria and herbicides, and poorly related to Gram-negative bacteria, *Actinobacteria,* and the evolution of time. The unamended soils were in the negative zone of PC2. The unamended soils were therefore less related to soil OC, microbial biomass, soil respiration and DHA than the GC-amended soils. The application of herbicides had no major impact because of these treatments' low shift in PC2. The individual or combined application of TSF and PSC in the GC-amended soils had a low impact in PC1, albeit with a clear decrease in PC2 scores compared to the Soil + GC control soil.

At 30 days after herbicide application, the unamended and GC-amended soils, untreated and treated with herbicides, recorded a similar shift to the positive zone of PC1, indicating herbicide dissipation, and a shift to Gram-negative bacteria and *Actinobacteria*. The unamended and GC-amended soils decreased their scores in PC2. This evolution indicated a decrease in soil microbial biomass and activity. However, both soils continued to record differences in PC2, where the unamended soils had lower scores. The presence of TSF, PSC, or a combination thereof, did not record a major shift from the control treatments (Soil and Soil + GC), so the impact of these herbicides after 30 days did not have a significant overall impact on microbial structure and activity.

At 100 days after herbicide application, the unamended and GC-amended soils presented a clear difference in the PCA analysis. The unamended soils (Soil, Soil + PSC and Soil + TSF) recorded a higher score in PC1 than the GC-amended soil. In contrast, the combination of herbicides in the unamended soil (Soil + TSF + PSC) recorded a similar value for the PC1 score as the GC-amended soils. Therefore, treatments

Table 2
Pearson correlation coefficients between relative percentage of Gram-negative and Gram-positive bacteria, *Actinobacteria* and fungi, soil dehydrogenase activity (DHA), soil respiration, soil biomass and organic carbon, percentage of remaining triasulfuron and prosulfocarb and ratio Gram-negative/Gram-positive bacteria. Significant correlations were denoted by asterisks and bold font.

	Gram −	Gram +	*Actinobacteria*	Fungi	DHA	Respiration	Biomass	OC	Triasulfuron	Prosulfocarb	Gram + /Gram −
Gram −	1										
Gram +	**−0.904*****	1									
Actinobacteria	**0.820*****	**−0.752*****	1								
Fungi	−0.113	−0.051	−0.233	1							
DHA	−0.247	**0.449***	−0.333	−0.025	1						
Respiration	**0.502***	**−0.487***	0.207	−0.032	0.001	1					
Biomass	−0.020	−0.184	−0.220	0.014	0.221	**0.521****	1				
OC	−0.053	0.165	−0.05	−0.248	**0.436***	0.306	**0.432***	1			
Triasulfuron	**−0.436***	**0.447***	−0.334	0.200	0.126	**−0.439***	−0.075	−0.055	1		
Prosulfocarb	−0.404	**0.452***	−0.388	0.089	0.255	−0.320	−0.113	−0.118	0.298	1	
Gram + /Gram −	**−0.942*****	**0.989*****	**−0.765*****	−0.007	0.385	**−0.518****	−0.169	0.084	**0.473***	**0.451***	1

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

Soil, Soil + PSC and Soil + TSF were more closely related to a high proportion of Gram-negative bacteria and *Actinobacteria* and low microbial biomass and activity than the combined herbicide treatment in Soil and Soil + GC. This means that the combination of TSF and PSC in the unamended soil changed the microbial structure compared to the individual application of these herbicides or to no application at all. However, the individual application of TSF or PSC did not produce a major shift in the microbial structure compared to the untreated soil (PC1). The relative low distance of unamended soils (Soil, Soil + TSF, Soil + PSC and Soil + TSF + PSC) along PC2 denotes low impact of these herbicides on soil microbial biomass and activity at the end of the assay. In the case of the GC-amended soils, the application of TSF or

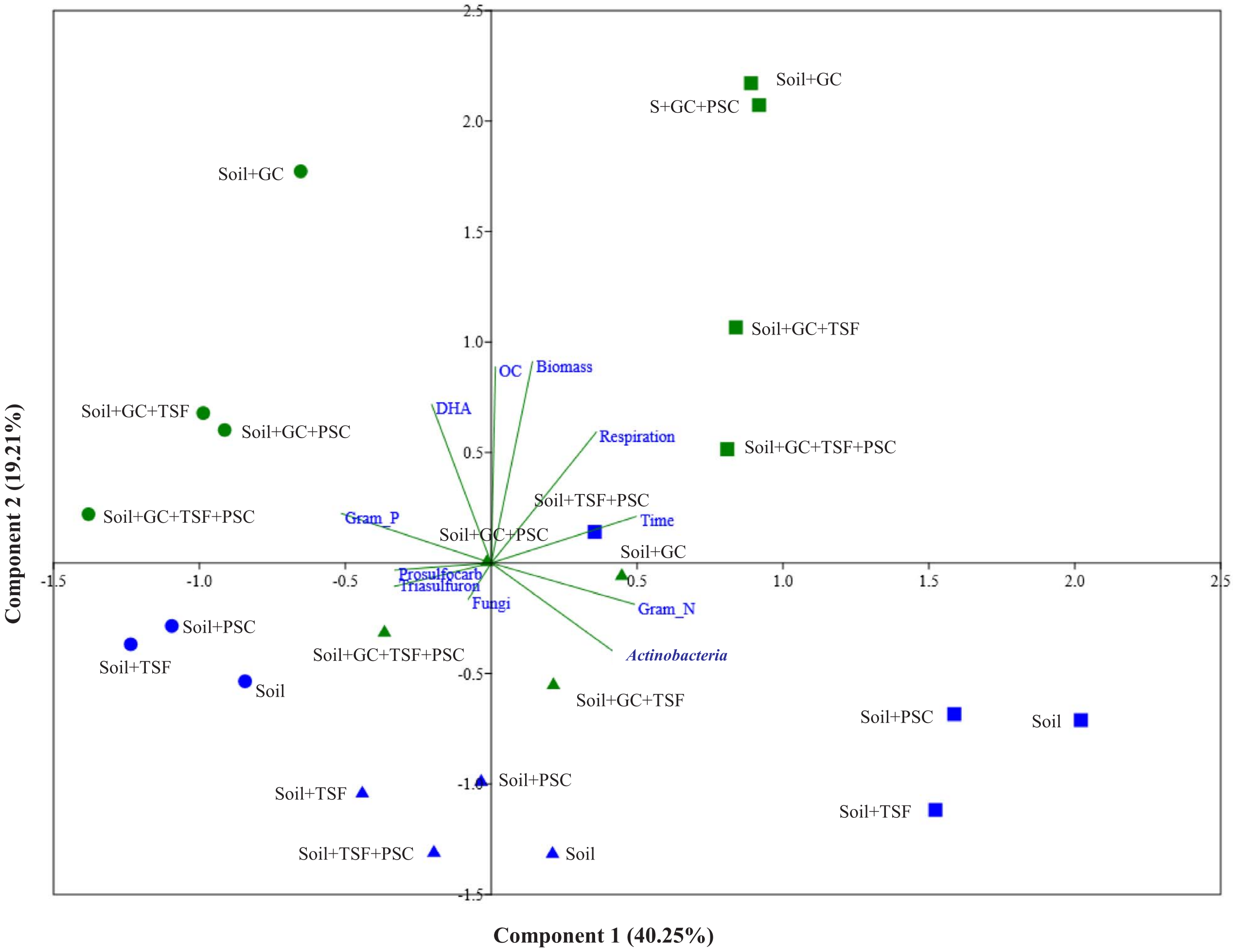

Fig. 6. Principal component analysis (PCA) showing loading scores for Gram-negative and Gram-positive bacteria, *Actinobacteria*, fungi, dehydrogenase activity, C-microbial biomass, soil respiration, soil organic carbon content (OC), percentage of remaining TFS and PSC and sampling time and scores of each treatment (GC: green compost; TSF: triasulfuron; PSC: prosulfocarb) and sampling time (0 days: circles; 30 days: triangles; 100 days: squares) on the two main principal components. Unamended and GC-amended soils were denoted by blue and green colors, respectively. Percent variability explained by each principal component is shown in parentheses after each axis legend ($n = 3$).

PSC impacted on PC2, related to microbial biomass and activity but not on PC1, related with the microbial structure. Soil + GC + PSC is very close to Soil + GC, indicating a similar evolution (Fig. 6B) and therefore low impact of PSC in the microbial activity on GC-amended soil. In contrast, the application of TSF (Soil + GC + TSF), and moreover the combined application of TSF and PSC (Soil + GC + TSF + PSC), clearly decreased the score of PC2 with respect to Soil + GC and Soil + GC + PSC, indicating negative impact on microbial activity. The application of TSF and the combination of TSF and PSC therefore impacted negatively on microbial biomass and activity at 100 days in GC-amended soil. Prosulfocarb's lower impact than TSF on soil microbial structure and activity could be related to the former's higher hydrophobicity (Table SI 1) and faster dissipation (Table 1), as well as to its volatile nature (Braun et al., 2017; Nunes et al., 2013), which minimized PSC availability to soil microorganisms.

Therefore, at the end of the assay, combination of TSF and PSC in unamended soil produced a shift of the microbial structure while individual application of TSF or combination of TSF and PSC in GC-amended soil produced negative effects on microbial biomass and activity but not microbial structure shift.

4. Conclusions

The simultaneous application of GC as an organic amendment and the herbicides TSF and PSC in an agricultural soil at field scale impacted on soil microbial activity and structure. The sulfonylurea herbicide TSF recorded a higher impact than the thiocarbamate herbicide PSC on soil microbial biomass and respiration. The combined application of TSF and PSC in an unamended soil produced a shift in the soil microbial structure. GC is useful for buffering the effects of herbicides on soil microbial biomass and activity, and reduces the shift in the soil microbial structure. However, despite the buffer effect of GC on microbial community towards herbicides, the combined application of TSF and PSC in GC-amended soil produced changes in soil microbial abundance and activity compared to the application of these herbicides individually or to no application at all. The use of GC is therefore recommended to minimize the impact of herbicides on soil microbiota, and furthermore reduce the risk of pollution by herbicide leaching. Finally, additional studies are also needed to evaluate the impact of additives (solvents and surfactants) present in commercial formulations of pesticides on the soil microbial communities. The possible negative effects of these compounds in pesticide formulations need to be evaluated according with the EU regulation (EC1107/2009) concerning the introduction of plant protection products on the market.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2018.03.004.

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Material Suplementario

SUPPLEMENTARY MATERIAL

Simultaneous application of two herbicides and green compost in a field experiment: Implications on soil microbial community

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Number of Tables: 2

Table S1.

Physicochemical characteristics of herbicides (PPDB, 2017).

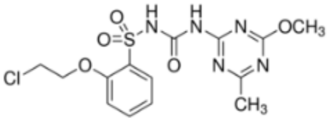
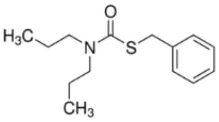
	Triasulfuron	Prosulfocarb
Chemical name (IUPAC)	1-[2-(2-chloroethoxy)phenylsulfonyl]-3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)urea	*S*-benzyl dipropyl(thiocarbamate)
Chemical structure		
Water solubility (mg L^{-1})	815 (20°C, pH 7)	13.0 (20°C, pH 6.1)
log Kow	-0.59 (20°C, pH 7)	4.48 (20°C, pH 7)
DT_{50} (laboratory) (days)	59.1	12.4
DT_{50} (field) (days)	38.5	9.8
pKa	4.64 (at 25°C)	-
Gus index	5.12	0.84

Table S2.

Properties of the unamended soil and green compost amended soil from experimental plots (0-10 cm)

Properties	Soil	Soil+GC
Soil texture	Sandy clay loam	Sandy clay loam
pH	7.81	7.13-7.39[†]
Bulk density (g cm^{-3})	1.39	1.00
OC (%)	1.30	2.21-2.36[†]
DOC (%)	0.002	0.015-0.019[†]
N (%)	0.14	0.23-0.25[†]
C/N	9.28	9.44-9.61[†]
$CaCO_3$ (%)	0.21	
Sand (%)	57.63	
Silt (%)	16.97	
Clay (%)	24.98	
Clay mineralogy	K, I, M[‡]	

[†] Value ranges correspond to mean values of three plots at different sampling times. [‡] K, kaolinite; I, illite; M, montmorillonite.

Experimental set up

The field experiment was conducted in an agricultural sandy clay loam soil (Typic Haploxerept) located in the experimental farm Muñovela belonging to the Institute of Natural Resources and Agrobiology of Salamanca, Spain (40º55’56”N latitude, 5º52’53”W longitude). Rainfall and air temperature were monitored over the 100 days of experimentation at a meteorological station installed on site. An experimental layout of randomized complete blocks was designed in February 2015 with eight treatments and three replicates per treatment (24 plots of 3 m × 3 m) corresponding to unamended soil (12 plots) and soil amended with GC at the rate of 120 t ha^{-1} on a dry weight basis (~ 11.6 t C ha^{-1}) (12 plots). Three unamended and three amended plots were treated with single commercial formulations of each herbicide (Logran® and Auros®), or a combined commercial formulation of both herbicides (Auros Plus®). Three more control plots (unamended and amended) did not receive any herbicide. Prior to soil amendment, the soil was tilled using a field cultivator, and then GC was manually mixed with the topsoil (0-20 cm) in each plot. The study was conducted in the cereal growing season, but the plots were left fallow.

Herbicides extraction and analysis

Duplicate subsamples of moist soil (6 g) from each plot were transferred to a glass tube, and extracted with methanol (12 mL). The samples were sonicated for 1 h, shaken at 20ºC for 24 h, and then centrifuged at 5045 g for 15 min and filtered to remove particles > 0.45 μm in a GHP Acrodisc filter (Waters Corporation). A volume of 8 mL was transferred to a clean glass tube and evaporated until dryness at 25ºC under a nitrogen stream using an EVA-EC2-L evaporator (VLM GmbH, Bielefeld, Germany). The residue was dissolved in 0.5 mL of methanol+formic acid (1%), and transferred to a HPLC glass vial for analysis.

The analysis of triasulfuron and prosulfocarb was performed by HPLC. The apparatus used was a Waters chromatograph (Waters Assoc., Milford, MA, USA), equipped with a model e2695 multisolvent delivery and autosampler system attached to a ZQ mass spectrometer detector (MS), with Empower software as the data acquisition and processing system. A Luna® 3 µm PFP(2) 100 Å (150 × 4.6 mm) column by Phenomenex (Torrance, CA, USA) was used at ambient temperature, and the mobile phase was acetonitrile:water+1% formic acid (70:30). The flow rate of the mobile phase was 0.4 mL min^{-1} and the sample injection volume was 10 µL. Detection involved monitoring the positive molecular ion [m/z] 402.8 (triasulfuron) and 252.4 (prosulfocarb) and the retention times were 6.1 min and 14.1 min, respectively. The matrix-based calibration standards were between 0.1 to 2.5 µg mL^{-1} for both herbicides, and the limit of detection (LOD) and limit of quantification (LOQ) were in the ranges 0.018-0.026 or 0.059-0.088 µg mL^{-1} for triasulfuron and 0.005-0.008 or 0.017-0.027 µg mL^{-1} for prosulfocarb in the unamended and amended soils, respectively. The method´s recoveries were determined by spiking three unamended and amended soil samples with analytical grade triasulfuron or prosulfocarb at similar concentrations to those applied in the field, and performing the extraction procedure as described above. The mean recovery values for a level spiking of 76.9 µg kg^{-1} (triasulfuron) and 3.46 mg kg^{-1} (prosulfocarb) were >80% for triasulfuron applied individually (Logran®) or in combined form (Auros Plus®), and >90% or >70% for prosulfocarb applied individually (Auros®) or combined form (Auros Plus®) for the unamended and GC-amended soils.

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CAPÍTULO 3

Resumen

CAPÍTULO 3: Recycling organic residues in soils as amendments: Effect on the mobility of two herbicides under different management practices

RESUMEN

La aplicación de residuos orgánicos en suelos para aumentar su contenido en materia orgánica (MO) es una buena opción para asegurar la producción sostenible de cultivos. La MO tiene una influencia directa en las propiedades físicas, químicas y microbiológicas de los suelos y, por lo tanto, en su fertilidad y en el desarrollo de las plantas por lo que su aumento en suelos sensibles a la degradación y a la erosión presenta un gran interés. Sin embargo, es necesario evaluar diferentes factores para optimizar el reciclaje de residuos orgánicos en suelos agrícolas, ya que pueden alterar el comportamiento de herbicidas cuando se aplican de manera conjunta en suelos agrícolas.

En este trabajo se ha estudiado la influencia de la dosis de enmienda aplicada en el suelo (~ 12 t C ha^{-1} y 40 t C ha^{-1}), diferentes condiciones de riego (sin riego y con riego adicional de 2.8 mm por semana), y aplicación repetida de herbicidas en la movilidad y persistencia de triasulfuron y prosulfocarb. Los herbicidas se aplicaron como una formulación comercial conjunta, Auros Plus®, en un suelo sin enmendar (S) y enmendado con compost vegetal (GC1 y GC2) en un experimento de campo. El diseño del experimento consistió en 24 parcelas de 3 m × 3 m y las dosis de los herbicidas aplicados fueron 11.25 kg i.a. ha^{-1} de prosulfocarb y 250 g i.a. ha^{-1} de triasulfuron (2.5 veces la dosis agronómica en ambos casos). La misma dosis se aplicó de nuevo cuando se alcanzó aproximadamente el 50% de su disipación (68 días).

Inicialmente, de acuerdo con las concentraciones residuales medidas en el perfil del suelo después de la primera aplicación de los compuestos, no se encontraron diferencias significativas entre los diferentes tratamientos y condiciones de irrigación en la movilidad del triasulfuron. El efecto del riego fue evidente un mes después de añadir los herbicidas y el efecto del tratamiento del suelo fue significativo dos meses después porque la persistencia de triasulfuron en S+GC2, con mayor dosis de enmienda, fue mantenida hasta el 50% de la cantidad aplicada. A este tiempo, las mayores cantidades residuales de triasulfuron observadas a lo largo del perfil del suelo se encontraron en los suelos enmendados a los que se añadió riego adicional.

En el caso del prosulfocarb, la influencia del GC fue significativa en su persistencia inicial en S+GC2, siendo mayor que en S+GC1 y S. Su presencia pudo

observarse en el perfil del suelo 13 días después de su aplicación, especialmente en S y S+GC1 al mismo tiempo que se registró una disipación cercana al 50% en S y S+GC1, y sólo de un 10% en S+GC2. Un mes después de su aplicación, el porcentaje de disipación aumentó en los suelos sin enmendar y enmendados, y dos meses después el porcentaje de prosulfocarb detectado fue <3% en los suelos enmendados y no se detectó en los suelos sin enmendar.

Un mes después de la segunda aplicación de los herbicidas, se observó un aumento de la disipación del triasulfuron con respecto a la primera aplicación. El aumento de la disipación puede ser debido a una degradación acelerada por parte de los microorganismos ya adaptados permitiendo un metabolismo más rápido del herbicida. Además, el incremento de temperatura media registrado (~10ºC) tras la segunda aplicación podría también haber acelerado el proceso de disipación. La movilidad del herbicida aumentó de igual manera después de la segunda aplicación. Se observaron concentraciones residuales del herbicida en el perfil del suelo S+GC2 debido a su adsorción por el suelo. La persistencia del triasulfuron (en superficie) se mantuvo cinco meses después de su aplicación repetida en suelos enmendados, aunque disminuyó con respecto a la observada tras la primera aplicación.

De manera similar al triasulfuron, la disipación del prosulfocarb después de la segunda aplicación fue más rápida que después de la primera y esto estuvo también relacionado con la adaptación previa de los microorganismos después de la primera aplicación y con las temperaturas más altas registradas durante el segundo periodo que favorecieron además una mayor volatilización del herbicida. Las cantidades registradas en el perfil del suelo indicaron la presencia del herbicida en todos los suelos alcanzando una profundidad de 40-50 cm, especialmente en el caso del suelo sin enmendar a pesar de la baja cantidad de agua recibida durante la segunda aplicación. Las concentraciones residuales remanentes en la superficie del suelo indican que la disipación disminuyó con el tiempo, pero no se alcanzó la desaparición total del compuesto en ningún suelo tras la segunda aplicación.

Los resultados indicaron que la aplicación de diferentes dosis de GC al suelo ofrece la posibilidad de lograr un balance entre las concentraciones residuales y el efecto sobre el suelo agrícola.

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Research article

Recycling organic residues in soils as amendments: Effect on the mobility of two herbicides under different management practices

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Herbicide
Repeated application
Green compost doses
Soil
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A B S T R A C T

The addition of organic residues to soil to increase its organic matter content is considered as a viable option for sustainable food production in soils sensitive to degradation and erosion. However, the recycling of these organic residues in agricultural soils needs to be previously appraised because they can modify the behaviour of pesticides when they are simultaneously applied in agricultural practices. This study evaluated the changes in the mobility and persistence of two herbicides, triasulfuron and prosulfocarb, after two repeated applications in field experimental plots in an unamended soil and one amended with green compost (GC) for seven months. Different factors were studied: i) soil without amendment (S), ii) soil amended with two doses of GC (~12 t C ha^{-1}, S + GC1 and 40 t C ha^{-1}, S + GC2), and iii) soils unamended and amended with different irrigation conditions: non-irrigated and with additional irrigation (2.8 mm per week). After the first application of herbicides, the results initially indicated no significant effects of soil treatments or irrigation conditions for triasulfuron mobility in agreement with the residual concentrations in the soil profile. The effect of irrigation was noted after one month of herbicide application and the effect of the soil treatment was significant after two months because the persistence of triasulfuron in S + GC2 was maintained until 50% of the applied amount. For prosulfocarb, the influence of soil amendment was significant for the initial persistence of the herbicide in S + GC2, higher than in S or S + GC1, in agreement with its adsorption constants for this soil. However, dissipation or leaching of the herbicide over time was not inhibited in this soil. After the repeated application of herbicides, the influence of the treatment of soils and/or irrigation was significant for the leaching and dissipation of both herbicides. The initial dissipation/degradation or leaching of herbicides was higher than after the first application, although persistence was maintained after five months of application in amended soils for triasulfuron and in unamended and amended soils for prosulfocarb. The results confirm that high doses of GC increased the persistence of both herbicides. This practice may offer the possibility of applying a tailored dose of GC to soil for striking a balance between residual concentrations and the soil agronomic effect.

1. Introduction

Management strategies are now being implemented for sustainable food production in soils susceptible to degradation and erosion. These strategies involve an increase in soil organic matter (OM) (Martins Gomes et al., 2018) because OM has a direct influence on the physical, chemical and microbiological properties of soils, and hence on soil fertility and plant development (Bastida et al., 2007; Tejada et al., 2009; Yazdanpanah et al., 2016). A possible option is the addition to the soil of amendments or organic wastes from different origins (urban, agricultural or industrial), which are currently generated in large quantities. The improvement in soil properties due to the OM content in these residues has been well documented, so its application as an organic amendment in agriculture is a common practice (Aranda et al., 2015; Bastida et al., 2015; Ferreras et al., 2006; Tejada and Gonzalez, 2008). In addition, the application of organic wastes represents an opportunity to increase the soil capacity for carbon sequestration, especially lignocellulosic organic wastes (Castellano et al., 2015; Hernandez et al., 2017). These residues have a more recalcitrant or less biodegradable nature than other residues, albeit with greater affinity to bind to the soil's mineral particles, increasing the stable soil carbon pool when protected against decomposition (Nicolás et al., 2017).

After being composted, the plant residues generated in the pruning carried out in parks and gardens have an OM content > 15% on dry weight and could be considered a more stable source of soil OM than that provided by other more biodegradable residues such as biosolids or

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sewage sludge. However, the recycling of these organic residues in agricultural soils has to be previously evaluated because they can modify the behaviour of pesticides when they are simultaneously applied in agricultural practices (Herrero-Hernández et al., 2011). Organic residues can be effective tools for controlling soil and water pollution by pesticides, but the agronomic efficiency of these compounds could be decreased by changing their adsorption, mobility, persistence or bioavailability. In this sense, investigations are necessary before the implementation of these products in agricultural soils to optimize their use in pursuit of pollution control and/or the agronomic effect of pesticides. Some studies related to this subject are reported in the literature for different types of pesticides (Jiang et al., 2016; Marín-Benito et al., 2009a, 2009b; Tejada and Benítez, 2017). In general, these studies are carried out under laboratory conditions, being rarely conducted under field conditions for a more realistic approach to this issue (Herrero-Hernández et al., 2015; Stipičević et al., 2015).

The evaluation of the effect of organic amendments in soils with cereal crops and its possible incidence on the behaviour of simultaneously applied herbicides is of special interest. Cereals are among the most important crops in world agriculture, with Spain devoting over five million ha to this crop. It is one of the crops with the largest amount of active ingredients available to be used as herbicide, and the competition between weeds and the crop will depend on, among other factors, the conditions of the environment and the soil. Compounds of the chemical groups sulfonamide and thiocarbamate, such as trisulfuron and prosulfocarb, are usually recommended for individual or joint use for the control of weeds in rainfed and irrigated cereal crops since provide a good control of weeds (Bajya et al., 2015; Cirujeda and Taberner, 2010; Knežević et al., 2010; Mehmeti et al., 2018).

Triasulfuron (2- (2-chloroethoxy) -N - [[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) amino] carbonyl] benzene sulfonamide) is a sulfonylurea with selective herbicide activity in the pre- and post-emergence control of broadleaf species in barley, oats and wheat. Triasulfuron has a high mobility in the soil due to its high solubility in water and low hydrophobicity (EC, 2000). Prosulfocarb (S-(phenylmethyl) dipropylcarbamothioate) is a systemic thiocarbamate with selective herbicide activity, being applied in early pre- and post-emergence against grasses and broadleaf weeds in crops such as long-cycle barley and wheat. It is a hydrophobic herbicide and has high adsorption, low mobility, and moderate persistence in soil (EFSA, 2007a).

In a previous work, we evaluated the behaviour of these herbicides, triasulfuron and prosulfocarb, in a short field experiment in an unamended soil and one amended with a low dose of green compost (GC) (Marín-Benito et al., 2018). The dissipation and mobility of herbicides were determined from concentrations obtained at various times in the soil surface and at different depths up to 50 cm. Variable effects of organic carbon (OC), dissolved organic carbon (DOC) from GC, soil water infiltration or formulation type (Logran®, Auros® and Auros Plus®) on the adsorption/persistence and leaching of herbicides in the soil profile were observed. The interest of these results is sustained considering that the leaching of herbicides from the soil surface shortens the duration of residual weed control and may contaminate groundwater. Furthermore, the amounts retained in the soil profile could exceed the recorded threshold for the sensitivities of susceptible species. The results of previous experiments were obtained after a single application of these herbicides and a single dose of GC. However, in practice, herbicides could be applied repeatedly to the soil-crop system for weed control with different soil amendment doses, and these practices can alter their degradation rate and persistence in soil, making it difficult to forecast their environmental impact.

Considering there are no studies on prosulfocarb mobility in soils in the literature, while the mobility of triasulfuron has been evaluated at field and laboratory scale (Stork, 1995; Weber et al., 1999), although in no case have they been carried out on soils amended with organic wastes, it is deemed of interest to expand the study of the behaviour of both herbicides under broader conditions. Both herbicides are usually applied as individual formulations or as a joint one when it comes to tackling a weed mixture. A good weed control of winter wheat (> 96.7%) has been reported with the herbicide combinations of prosulfocarb plus triasulfuron (Knežević et al., 2010).

Accordingly, the objective of this paper was to expand the study of the mobility of triasulfuron and prosulfocarb using a joint formulation (Aurus Plus®) in an agricultural field devoted to cereal cultivation under different conditions: i) soil without amendment, ii) soil amended with two doses of green compost, and iii) soils unamended and amended with different irrigation conditions (non-irrigated and with additional irrigation). These factors were studied in field experimental plots in two time periods (2 + 5 months) during which repeated doses of herbicides were applied, and the results involved the physical, chemical and physicochemical properties of soils.

2. Materials and methods

2.1. Herbicides

The commercial formulation of triasulfuron + prosulfocarb (Auros Plus®) (Syngenta Agro S.A., Madrid, Spain) was used in the study. Analytical standards of both compounds were purchased from PESTANAL® (purity > 98.9%) (Sigma Aldrich Química S.A., Madrid, Spain). Water solubility is 815 and 13.0 mg L^{-1} and log Kow is −0.59 and 4.48 for triasulfuron and prosulfocarb, respectively (PPDB, 2018). The chemical structure and general characteristics are included in Table S1 in Supplementary material.

2.2. Green compost residues

Two composted organic residues of vegetal origin (< 5 mm) generated from the pruning of plants and trees in parks in the city of Salamanca (NW-Spain) were used. They were supplied by the City Council (GC1) and by the nursery "El Arca" in Salamanca (Spain) (GC2). Their main physicochemical characteristics on a dry weight basis were determined as indicated in Marín-Benito et al. (2018), and they are pH 7.33 and 7.58, OC% 9.8 and 24.1 and N% 1.04 and 1.10, respectively, for GC1 and GC2.

2.3. Experimental set-up

The field assay was conducted in plots located in the Muñovela experimental farm belonging to the Institute of Natural Resources and Agrobiology of Salamanca, Spain (latitude 40º54′15″ N, longitude 5º46′31.51″ W). These plots were located in the same experimental field (sandy clay loam soil, Typic Haploxerept) (Soil Survey Staff, 2010) where we carried out a previous experiment in 2015 (Marín-Benito et al., 2018). In February 2016, the experimental layout of randomized complete blocks (18 plots of 3 m × 3 m) was designed as follows: unamended soil (S, 6 plots), soil amended with GC1 at the rate of 120 t ha^{-1} on a dry weight basis (~12 t C ha^{-1}) (S + GC1, 6 plots) and soil amended with GC2 at the rate of 180 t ha^{-1} on a dry weight basis (~40 t C ha^{-1}) (S + GC2, 6 plots) (Fig. 1). GC1 and GC2 amendments were used by availability effect to get different OC content in plots. They were incorporated into the 20-cm topsoil layer by using a rototiller. Three plot replicates per treatment received only natural rainfall (NI), and a further three replicates per treatment received additional sprayer irrigation (I, 2.8 mm per week).

Eighteen unamended and amended plots were treated with the combined commercial formulation of both herbicides (Auros Plus®). Water herbicide solutions were applied manually using a backpack sprayer (volume of 5 L) shortly after the soil was amended. The doses jointly applied to the plots were 11.25 kg a.i. ha^{-1} of prosulfocarb as Auros® 80% and 250 g a.i. ha^{-1} of triasulfuron as Logran® 20%, corresponding to 2.5 times the maximum agronomic dose for both herbicides recommended for heavy soils with a greater adsorption capacity.

Datum: ETRS89
Latitud: 40º 54' 15.01" N
Longitud: 5º 46' 31.51" W
Huso UTM: 30
Coord. X: 266.232,00
Coord. Y: 4.531.828,03
Nivel: 19

Fig. 1. Location of plots in the experimental farm.

The increase in the soil's capacity for adsorbing the herbicides after an organic amendment supports the use of doses higher than those recommended to maintain the efficacy of the compounds (Worrall et al., 2001). Two repeated applications of herbicides were carried out in this experiment; the first dose was applied in March 2016, and the second one was applied once the dissipation of herbicides had surpassed 50% in May 2016 (68 days after the first application).

The estimated concentrations applied were 0.1923 mg kg^{-1} and 8.653 mg kg^{-1} for triasulfuron and prosulfocarb, respectively, calculated considering a soil density of 1.3 g cm^{-3} and a depth of 10 cm. However, the averaged concentrations measured in soil samples to check the dosage applied to soil ranged from 0.169 to 0.270 mg kg^{-1} and 5.088–8.794 mg kg^{-1} for triasulfuron and prosulfocarb, respectively, in different plots of unamended and amended soil after the first application. After the second application, the measured concentrations ranged from 0.257 to 0.291 mg kg^{-1} and 6.857–8.350 mg kg^{-1}for triasulfuron and prosulfocarb, respectively.

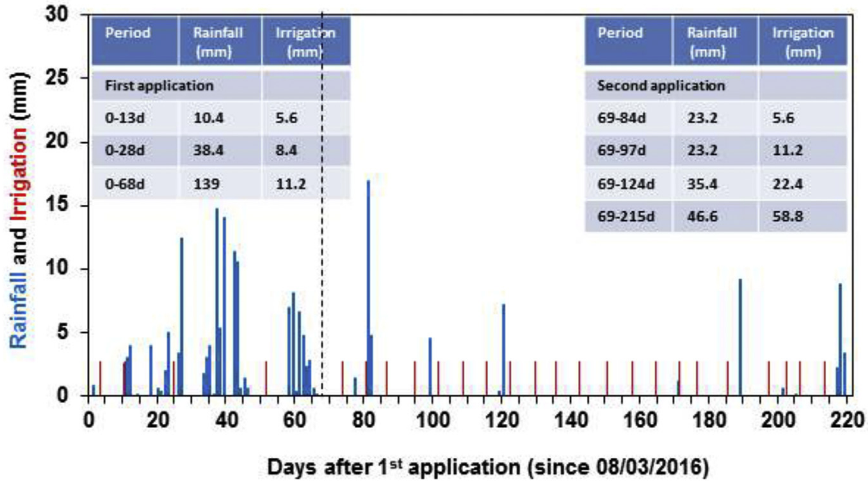
Rainfall and air temperature were monitored over the 215 days of experimentation by a weather station located on site. Air temperature ranged from 1.7 °C to 14.6 °C and from 10.5 °C to 26.3 °C during the first and the second period of the application, 68 and 147 days, respectively. Cumulative rainfall and rainfall + irrigation were 139–150 mm and 46.6–105 mm during the first and the second period of the application, respectively (Fig. 2).

2.4. Soil sampling and sample processing

Soil sampling was carried out at 13, 28 and 68 days after the first application of herbicides, and after 15, 28, 55, and 147 days after the second application of herbicides (corresponding to 84, 97, 124 and 215 days from the initial application) to study the influence of repeated doses on the downward mobility and persistence of herbicides in the soil samples. Five soil cores from 0 to 50 cm were collected in each plot and then sectioned into five segments of 10 cm each, and composite samples of five cores were transferred to polypropylene bottles. All the samples were transported to the laboratory in portable refrigerators. The moisture content of the bulk sample for each 10-cm soil layer was gravimetrically determined by weight difference, measuring the soil sample mass before and after drying at 110 °C for 24 h. The characteristics of soil samples, previously air-dried overnight if necessary and sieved (< 2 mm), were determined by standard methods, as indicated in Marín-Benito et al. (2018). Dissolved organic carbon (DOC) was determined in soil/Milli-Q ultrapure water extracts (1/2 w/v ratio) after shaking (24 h at 20 °C), centrifugation (20 min at 10000 rpm), and filtering (Minisart NY 25 filter 0.45 µm, Sartorius Stedim Biotech, Germany) using a Shimadzu 5050 (Shimadzu, Columbia, MD, USA) organic carbon analyzer (Table 1). The pH, OC and DOC values were determined at different times after the application of herbicides to evaluate their influence on the behaviour of herbicides (Table 1 and Fig. S1 in Supplementary material). The adsorption capacity of triasulfuron and prosulfocarb by each 10-cm soil layer was determined as a single-point concentration (10 µg mL^{-1}) at the beginning of the experiment, and the distribution coefficients, Kd (mL g^{-1}), were calculated as indicated in Marín-Benito et al. (2018) (Table S2 in Supplementary material).

2.5. Herbicide extraction and analysis

The experimental method of herbicide extraction and analysis was as indicated in Marín-Benito et al. (2018). Briefly, duplicate subsamples of moist soil (6 g) from each section (0–10 cm) of soil cores were extracted with methanol (12 mL) and underwent sonication (1 h), shaking at 20 °C (24 h), and centrifugation (15 min). A volume of 8 mL was then evaporated until dryness at 25 °C under a nitrogen stream using an EVA-EC2-L evaporator (VLM GmbH, Bielefeld, Germany). The residue was dissolved in 0.5 mL of methanol + formic acid (1%) and transferred to a HPLC glass vial for analysis. The analysis of triasulfuron and prosulfocarb was performed in a Waters chromatograph (Waters Assoc.,



Period	Rainfall (mm)	Irrigation (mm)
First application		
0-13d	10.4	5.6
0-28d	38.4	8.4
0-68d	139	11.2

Period	Rainfall (mm)	Irrigation (mm)
Second application		
69-84d	23.2	5.6
69-97d	23.2	11.2
69-124d	35.4	22.4
69-215d	46.6	58.8

Fig. 2. Rainfall and irrigation evolution over time of experiment.

Table 1
Properties of the soils unamended and amended with green compost (GC1 and GC2) from experimental plots.

Properties	Soil depth (cm)				
	0–10	10–20	20–30	30–40	40–50
Soil texture	Sandy clay loam	Sandy clay loam	Sandy clay loam	Sandy clay loam	Sandy clay loam
pH	7.10–6.57^{a}	7.18–6.78	7.29–6.76	7.36–7.22	7.43–7.67
	7.69–7.35^{b}	7.50–7.69	7.29–7.59	7.38–7.67	7.38–7.65
	7.24–7.34^{c}	7.26–7.11	7.21–6.76	7.45–7.03	7.56–7.32
OC (%)	1.41–0.95^{a}	1.17–0.88	0.92–0.86	0.75–0.68	0.65–0.57
	2.29–1.89^{b}	1.74–1.24	1.02–0.84	0.76–0.71	0.67–0.49
	5.87–3.34^{c}	2.16–1.28	1.04–0.85	0.87–0.63	0.66–0.57
DOC (mg g^{-1})	0.06–0.02^{a}	0.11–0.03	0.09–0.03	0.08–0.03	0.04–0.05
	0.08–0.07^{b}	0.09–0.04	0.04–0.04	0.04–0.03	0.03–0.03
	0.32–0.19^{c}	0.07–0.04	0.05–0.02	0.02–0.02	0.03–0.02
N (%)	0.12–0.10^{a}	0.11–0.07	0.08–0.07	0.06–0.07	0.06–0.06
	0.19–0.22^{b}	0.12–0.16	0.09–0.10	0.08–0.07	0.13–0.06
	0.43–0.41^{c}	0.16–0.15	0.09–0.09	0.07–0.07	0.07–0.06
C/N	11.4–9.04^{a}	11.0–12.0	11.2–11.8	10.8–10.0	10.3–9.42
	11.9–8.93^{b}	14.6–7.61	11.3–8.63	9.30–10.2	5.30–7.97
	11.1–8.11^{c}	13.3–8.93	11.5–10.8	12.2–10.5	10.1–9.26
CaCO$_3$ (%)	0.21^{a}	0.18	0.20	0.22	0.22
Sand (%)	57.63^{a}	58.22	59.15	55.63	51.04
Silt (%)	16.97^{a}	16.95	16.95	16.77	17.60
Clay (%)	24.98^{a}	25.25	25.25	29.10	34.72
Clay mineralogy	K, I, M^{d}	K, I, M	K, I, M	K, I, M	K, I, M

Note: The ranges of values included for some parameters correspond to the variation of mean values of these parameters in three replicate plots between 13 and 215 days.

[a] Unamended soil.

[b] Soil amended with GC1 at 120 t ha^{-1}.

[c] Soil amended with GC2 at 180 t ha^{-1}.

[d] K, kaolinite; I, illite; M, montmorillonite.

Milford, MA, USA), equipped with a model e2695 multisolvent delivery and autosampler system attached to a ZQ mass spectrometer (MS) detector. Detection involved monitoring the positive molecular ion [m/z] 402.8 [M+H]$^{+}$ (triasulfuron) and 252.4 [M+H]$^{+}$ (prosulfocarb), and the retention times were 6.1 min and 14.1 min, respectively. The method's recoveries were determined by spiking three unamended and amended soil samples with triasulfuron and prosulfocarb at similar concentrations to those applied in the field, and performing the extraction procedure as described above. The mean recovery values were > 80% for both compounds in the unamended and GC-amended soils. The amounts of triasulfuron and prosulfocarb extracted from soils were not corrected for recovery values because matrix-matched calibration method was used.

2.6. Data analysis

Analysis of variance (ANOVA) was used to evaluate the effects of the different soil treatments on herbicide mobility. Standard deviation (SD) was used to indicate variability among replicates. Fisher's least significant difference (LSD) method, at a confidence level of 95%, was determined with SPSS Statistics 22.0 software for Windows (SPSS Inc. Chicago, USA).

3. Results

3.1. Soil characteristics

The values of pH, OC and DOC were determined for soils with different treatments (Table 1 and Fig. S1 in Supplementary material). The pH mean values of soils with different treatments were similar at the beginning (13 days) and at the end (215 days) of the experiment (7.32–7.28), although a significant decrease was recorded after 84–97 days of the application of herbicides (6.96–7.14) (LSD = 0.096, $p < 0.05$). However, significant differences were observed between the pH values of soils with different treatments (6.94–6.99 (S), 7.04–7.20 (S + GC2) and 7.31–7.47 (S + GC1)) or between different depths of soils (6.97–7.20 up to 10–30 cm and 7.39 up to 50 cm) (LSD = 0.088). Changes in the pH of amended soils were noted when additional irrigation was applied.

The soil OC content increased through the addition of organic residues (Table 1 and Fig. S1 in Supplementary material). The OC contents ranged between 1.41 and 0.95% for the unamended topsoil for all experimental times, and these contents increased with the application of GC up to two times in the S + GC1 (2.29–1.89%) and up to five times in the S + GC2 (5.87–3.34%). The soil OC content significantly increased up to 20–30 cm in the soil profile after the application of organic residues (LSD = 0.285, $p < 0.05$), while there were no differences between OC contents below 30 cm in the soil profile in any soil, and the OC contents ranged between 0.67 and 0.49% over the 215 days of experimentation. The increase in soil OC content due to the effect of GC1 was maintained in the different depths throughout the experimentation (seven months). However, the soil OC content after the application of the GC2 showed a higher decrease than after the application of the GC1, with the initial OC content in the topsoil decreasing ≈1.5 times by the end of the experiment, possibly due to the oxidation and/or mobility of the most labile forms of carbon. Despite this decrease, a significant increase ($p < 0.05$) in the OC content in the 0–10 cm and 10–20 cm layers was recorded in the amended soils with respect to the unamended soils, indicating that the application of the GC to the soil could improve soil properties over the long term. No significant changes in soil OC contents due to irrigation were observed.

The application of GC gave rise to an increase of DOC in the 0–10 cm layer of the S + GC2. The DOC content of the topsoil was 0.06 mg g^{-1} and remained almost constant until the end of the experiment. The DOC content increased up to 5–10 times after the addition of the GC2 to the soil, but the increase was only up to two times after the addition of GC1 as regards the unamended soil (Table 1 and Fig. S1 in Supplementary material). The highest mean values of DOC in all the soils were recorded after 13–84 days from the initial time, and the DOC content decreased ≈2 times at the end of the experiment in the 10–20 cm layer of the S + GC2. At this depth, only the mean values of DOC of S + GC2 without irrigation or with irrigation were significantly different to those from S and S + GC1 (LSD = 0.076, $p < 0.05$). The DOC may have been leached out of the S + GC2 soil profile, especially under conditions of pH > 7 for the soil studied, as reported by some authors (Temminghoff et al., 1997), and this process might affect the mobility of the herbicides applied.

3.2. Mobility of triasulfuron from Auros Plus® formulation

The amounts of triasulfuron in the soil profile (0–50 cm) were determined at different times after the first application (13, 28 and 68 days) and the second one (15, 28, 55 and 147 days) in S, S + GC1 and S + GC2, with and without additional irrigation (Fig. 3).

After 13 days of the first triasulfuron application, the remaining amounts in the 0–10 cm layer were > 70% in the S and S + GC2, and > 50% in the S + GC1 taking the amounts initially determined as the reference of application. In the 10–20 cm layer, the remaining amounts decreased below 5%, 8% and 2% in the S, S + GC1 and S + GC2, respectively. The amounts of triasulfuron in the topsoil (0–10 cm) were the highest in the soil profile, and there were no significant differences between the mean amounts detected in the other layers. The lowest mean amount of triasulfuron was detected in the S + GC1 profile, and the highest in the S profile (LSD = 0.017, $p < 0.05$). The presence of triasulfuron was detected in all the layers of the soil profile with different treatment or irrigation conditions in decreasing amounts up to 40–50 cm (< 0.0075 μg g^{-1}).

The distribution pattern of triasulfuron in the soil profile was similar

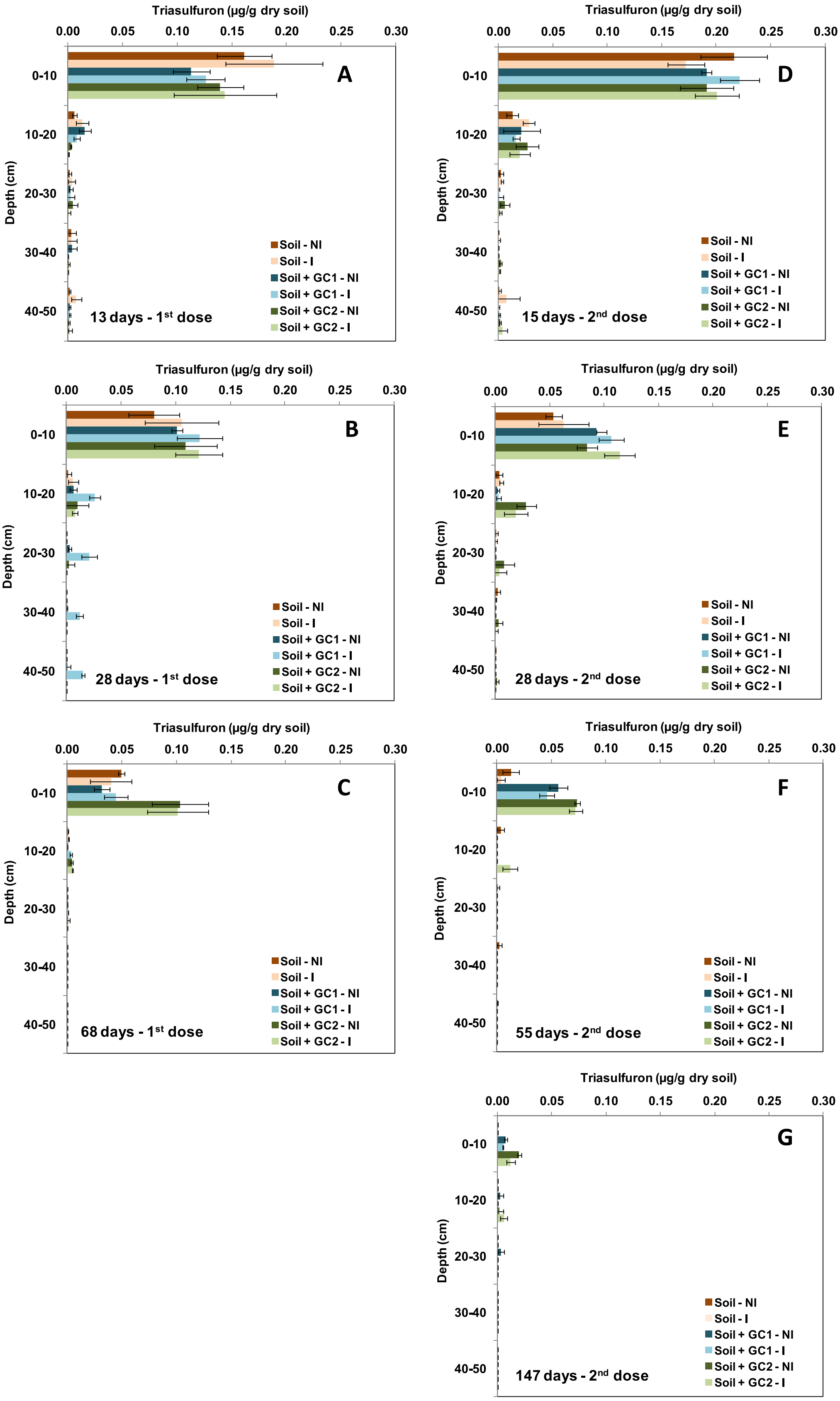


Fig. 3. Distribution profiles of triasulfuron in soil unamended and soil amended with green compost (GC1 and GC2) with non-irrigation (NI) and with additional irrigation (I) at different sampling times after the herbicide application (left graphs (A–C) correspond to the first application and right graphs (D–G) correspond to the second application). Error bars represent the standard deviation of mean values of plots treated (n = 3).

after 28 and 68 days of treatment, although the mean residual concentrations decreased in all the soils and layers (LSD = 0.009, $p < 0.05$). The herbicide concentration in the 0–10 cm layer decreased especially in S (28 and 68 days) and in S + GC1 (68 days), but the decrease in S + GC2 was not significant. The effect of GC2 on the persistence of triasulfuron in this soil appeared over time. It is worth noting a significant increase in the triasulfuron concentration recorded in S + GC1-I after 28 days in the 10–20 cm, 20–30 cm, and 40–50 cm layers. The recorded amounts of triasulfuron in the deeper layers of S + GC1 were significantly different to those found in the other soils, and herbicide amounts in the 40–50 cm depth were detected only in S + GC1-I. At this time, the highest mean amounts were recorded in the profile of the amended soils with the application of additional irrigation (S + GC1-I > S + GC2-I > S + GC2 > S + GC1 > S-I > S) (LSD = 0.007, $p < 0.05$). These differences were not observed after 68 days among soils with different treatments in soil layers under 0–10 cm, although a percentage close to 50% was detected in the topsoil of S + GC2, and traces of triasulfuron were found in amended soils up to 20–30 cm depth (< 0.0007 μg g^{-1}) in S + GC2 and 30–40 cm depth in S + GC1 (0.0005 μg g^{-1}).

The second application of triasulfuron was carried out when the residual concentration of herbicide was in the 15–55% range. The amounts remaining after 15 days were > 67%, > 70% and > 65%, in the soils S, S + GC1 and S + GC2, respectively, as regards those found immediately after the second application. The highest residual concentrations were also found in the 0–10 cm soil layers and were close to those found in the first application of the herbicide. However, an increase in the residual concentrations in the 10–20 layer was found compared to the first application, being significantly higher than those in the other soil profile layers (LSD = 0.011, $p < 0.05$) for soil samples with different treatments. The soil treatment had no influence on the levels of triasulfuron found at this time, and the presence of herbicide was also detected in all the soil profile layers of unamended or amended soils in decreasing amounts up to the 40–50 cm layer (< 0.0078 μg g^{-1}).

The distribution pattern of triasulfuron in the soil profile after 28 and 55 days was similar to that determined at 15 days, although the residual concentrations were lower. After 28 days, there were also differences between the residual concentrations in the soils, and the effect of the amendment and the application of additional irrigation were also significant, as in the first application. The detected amounts decreased according to the order S + GC2-I > S + GC2 > S + GC1-I > S + GC1 > S-I > S (LSD = 0.015, $p < 0.05$), and triasulfuron was detected only in the S + GC2 up to 40–50 cm depth (< 0.002 μg g^{-1}). Similar to the first application, differences between soil samples and layers below 0–10 cm were not observed after 55 days of treatment. The mean amounts found throughout the soil profile were not significantly different, and only traces of triasulfuron were found up to 10–20 cm depth (< 0.0012 μg g^{-1}) in S + GC2-I and to 30–40 cm depth in S (0.0003 μg g^{-1}). After 147 days, the residual concentrations of triasulfuron decreased drastically in the 0–10 cm layer, and no residual amounts of triasulfuron were detected in the unamended soil, and only traces of the herbicide were detected in S + GC1 up to 20–30 cm depth (0.003 μg g^{-1}) and up to 10–20 cm depth in S + GC2-I (0.006 μg g^{-1}) and S + GC2 (0.002 μg g^{-1}). The residues of triasulfuron indicated a lower persistence of herbicide in unamended and amended soils after repeated herbicide application.

3.3. Mobility of prosulfocarb from Auros Plus® formulation

The amounts of prosulfocarb in the soil profile (0–50 cm) at different times were recorded after the first application (13, 28 and 68 days) and the second one (15, 28, 55 and 147 days) in S, S + GC1 and S + GC2 with and without additional irrigation (Fig. 4).

After 13 days of the first herbicide application, the remaining amounts in the 0–10 cm layer were > 47%, > 48% and > 90%, and < 5%, < 9% and < 0.5% in the 10–20 cm layer in S, S + GC1 and S + GC2, respectively, taking as reference the amounts initially determined. Only the amounts found in the 0–10 cm layers were statistically different, and no significant differences were found between the amounts detected in the other layers of the soil profile. The influence of the soil treatment and/or the application of additional irrigation was not significant in the mean amounts found in the soil profile, although the highest amount of prosulfocarb in the topsoil was recorded in S + GC2. The presence of prosulfocarb was detected in all the soil profiles up to 40–50 cm depth in amounts < 0.2020 μg g^{-1}.

After 28 and 68 days, the distribution pattern of prosulfocarb in the soil profile was similar to that determined after 13 days of treatment, but the residual concentrations found at 68 days were lower than at 13 and 28 days (LSD = 0.565, $p < 0.05$). The highest herbicide concentration recorded in the 0–10 cm layer decreased significantly after 28 days, especially in S and S + GC1 and regardless of the application of additional irrigation. This decrease was more pronounced after 68 days in the amended soils, with no influence of additional irrigation, and in unamended soil where the dissipation of prosulfocarb was total and no herbicide was detected. After 28 days, traces of the compound were detected up to 20–30 cm in the unamended soil and up to 40–50 cm in the amended soils (≤0.032 μg g^{-1} in S + GC1 and ≤0.0126 μg g^{-1} in S + GC2). After 68 days, traces of the compound were detected up to the 40–50 cm depth (≤0.002 μg g^{-1}) in the amended soils (S + GC1 and S + GC2).

The second application of prosulfocarb was carried out when the dissipation of the herbicide was total (S) or almost total (< 1.5% in S + GC1 and < 3.1% in S + GC2). The amounts of prosulfocarb remaining in S, S + GC1 and S + GC2 after 15 days of the second application varied in the ranges > 6%, > 21% and > 31%, respectively, of that found immediately after its application. Similar to the first application, the highest residual concentrations were found in the 0–10 cm layer of the soil profile, and the residual concentrations of the herbicide in the deeper layers of the soil profile were not statistically different (LSD = 0.487, $p < 0.05$) for soil samples with different treatments. The presence of prosulfocarb was detected in all the layers of the soil profile in decreasing amounts for all soil treatments. Residues of prosulfocarb were detected up to 40–50 cm depth in amounts ≤0.1615 μg g^{-1}. Similar to the first application, no significant differences were found between the mean prosulfocarb amounts in the soil profiles with the different treatments, or with the application of additional irrigation.

The distribution of prosulfocarb in the soil profile 28 and 55 days after the second application was similar to that determined at 15 days, although the residual concentrations were lower, and only the amounts detected in the 0–10 cm layer were significantly different. However, after 28 days, the residual concentrations detected in all S + GC2 layers with or without additional irrigation were significant. The presence of GC2 favoured the leaching of prosulfocarb throughout the soil profile. This effect disappeared after 55 days, and only herbicide traces under the 0–10 cm layer were detected in all the soils. The herbicide amounts were greater in the amended soils (S < S + GC1 < S + GC2), and similar in S + GC2 to that recorded at 55 days, but they always diminished after the application of additional irrigation. After 147 days, the residual concentrations of repeatedly applied prosulfocarb decreased significantly in the 0–10 cm layer, following the same pattern as after 55 days, but residual concentrations were recorded in all the soils and in all soil profiles. It should be noted that residual concentrations of prosulfocarb were higher after 147 days than those obtained 68 days after the first application of the herbicide. The results indicate an increase in the persistence of prosulfocarb in the unamended and amended soils by repeated application of herbicide over time.

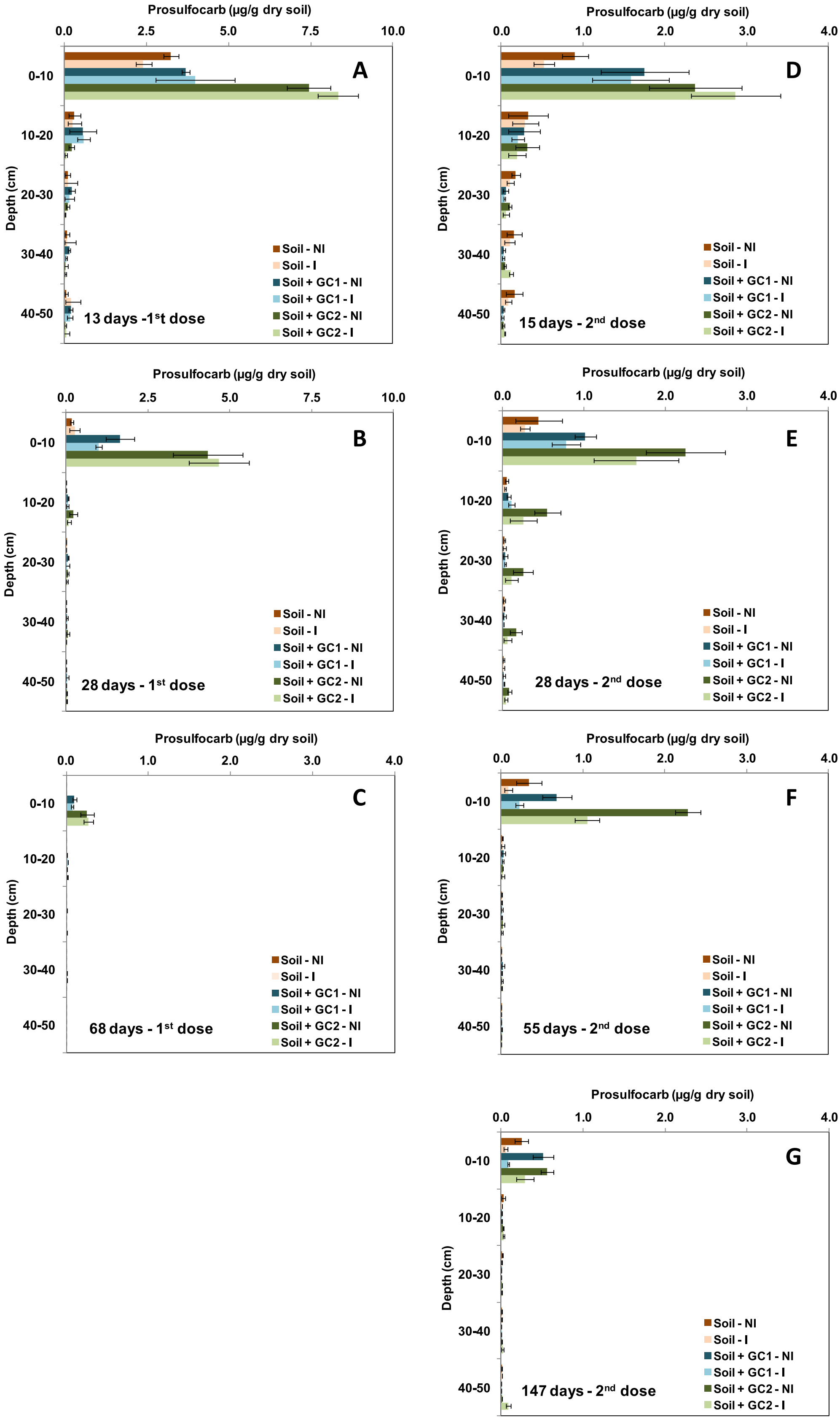


Fig. 4. Distribution profiles of prosulfocarb in soil unamended and soil amended with green compost (GC1 and GC2) with non-irrigation (NI) and with additional irrigation (I) at different sampling times after the herbicide application (left graphs (A–C) correspond to the first application and right graphs (D–G) correspond to the second application). Error bars represent the standard deviation of mean values of plots treated (n = 3).

4. Discussion

4.1. Triasulfuron mobility: effect of organic amendment and irrigation

Triasulfuron mobility was initially observed in the soil profile for all soil treatments. This behaviour is to be expected because sulfonylureas are weak acids and adsorption decreases when soil pH is higher than its pKa (4.64) (Pusino et al., 2003) increasing its mobility. In our experiment, the pH of the 0–10 cm layer was higher than 7, i.e., pH was higher than pKa, and this factor could increase the mobility of the herbicide. In fact, a significant correlation coefficient ($r = -0.863$, $p < 0.05$) was found between triasulfuron amounts in the 0–10 cm layers and soil pH after 13 days of treatment. However, this significant correlation was not observed between the residual concentrations in the soil profile and pH values for each soil, indicating the influence of other factors on herbicide mobility. After one month of the application, the dissipation of the compound was higher in the unamended soil than in the amended soils, with mobility in the soil profile being more significant in these soils. The effect of a rainfall event of 12.4 mm at 27 days, along with the additional irrigation applied, could have affected the mobility observed in S + GC1. Similar changes were also observed after two months of treatment, and the highest amounts of herbicide at this time were found in the surface horizon of S + GC2 with herbicide traces detected up to 20–30 cm (S + GC2 and S + GC1) and 30–40 cm (S + GC1). Herbicide leaching depended on soil treatment and rainfall and/or additional irrigation received after the first application of the herbicide over time (Fig. 2). A rapid transport into deep soil layers by the irrigation flow has been reported for triasulfuron and other different pesticides (Anyusheva et al., 2016; Salazar-Ledesma et al., 2018; Sarmah et al., 2000). Rainfall events at this time were more important for the transport of herbicide than the additional irrigation, although these events did not affect the dissipation of herbicide in S + GC2 to any greater extent.

The increase in the OC content of S + GC2 in the 0–10 cm layer could explain the persistence of more than 50% of the amount of herbicide initially applied in the soil after two months, indicating a slower dissipation of triasulfuron. A significant correlation between the residual concentrations of herbicide and the OC content of soils throughout the soil profile was found for S + GC2 ($r \geq 0.968$, $p < 0.01$) and S + GC1 ($r \geq 0.927$, $p < 0.05$) even though the OC content decreased significantly in the soil profile. This correlation was not significant for the unamended soil. The herbicide's behaviour is also consistent with the higher adsorption coefficients (Kd) for S + GC2, with the highest OC content (S, 0.31 ± 0.01 mL g^{-1}, S + GC1, 0.38 ± 0.09 mL g^{-1} and S + GC2, 0.67 ± 0.03 mL g^{-1}) (Table S2 in Supplementary material). Similar effects were observed for triasulfuron and other sulfonylureas in soils amended with other organic residues (Delgado-Moreno and Peña, 2008; Said-Pullicino et al., 2004). In addition, herbicide leaching may also be modified by structural changes in soil aggregate stability, porosity, hydraulic conductivity, water holding-capacity, etc. induced by the increase in OC content (Ingelmo Sánchez and Rubio Delgado, 2008; Yazdanpanah et al., 2016). In our study, a higher water holding-capacity of the topsoil in S + GC2 may be responsible for a lower water percolation in the soil profile.

The mobility of triasulfuron in the amended soils detected over the time of application of the herbicide could be explained by the mobilizing effect of the herbicide caused by the DOC in the soil profile considering a pH > 7 for the amended soils. At the basic pH shown by the amended soils (Fig. S1 in Supplementary material), the DOC is more easily mobilized and could favour the leaching of triasulfuron, even though the hydrophobicity of triasulfuron was not high enough to explain its adsorption by the DOC in solution, as indicated in the literature for other compounds of similar character (Marín-Benito et al., 2012; Rodríguez-Liébana et al., 2018; Rodríguez-Liébana and Peña, 2018). However, the DOC content of S + GC1 is relatively low for explaining the mobility of triasulfuron after 28 days. This herbicide mobility in S + GC1, but not recorded in S and S + GC2, could be due to the existence of random preferential flows in the soil within the individual plots because of its clay content ranging from 25% to 34% in the soil profile, which would facilitate the rapid leaching of the herbicide. In fact, cracks were noted in the S + GC1 plots after additional irrigation was applied. A similar behaviour was explained in previous studies (Sarmah et al., 2000; Anyusheva et al., 2016; Jarvis, 2007; Koestel and Jorda, 2014). The effect, observed only in the S + GC1-I, could not be explained by the greater amount of water applied to irrigated soils. The presence of DOC in the S + GC1 profile as a mobilizing effect of triasulfuron could not be considered because there was no difference between DOC of S and S + GC1 despite the fact that the higher pH of S + GC1 could mobilize the DOC to a greater extent.

4.2. Triasulfuron mobility: effect of repeated doses

After 15 days of treatment, no significant relationship was found between residual concentrations of triasulfuron in the 0–10 cm layers and the pHs of different soils, even though the pH values in the soil profile were higher for amended soils than for the unamended one. The residual concentrations detected in the 10–20 cm layer for all the soils at this time could possibly be influenced by the heavy rainfall recorded (17 mm) in the days prior to soil sampling and herbicide determination.

An increase in herbicide dissipation was observed one month after the repeated application with respect to the first application, consistent with the amounts of triasulfuron found in the topsoils. An accelerated degradation due to microbial adaptation leading to faster metabolism could explain this behaviour, as reported for other compounds (Fang et al., 2018; Singh et al., 2002). The degradation of the herbicide could also be due to the higher air temperatures recorded in the second part of the experiment (~10 °C higher). It is well known that the degradation rate of pesticides in soil depends heavily on the soil moisture and temperature. EFSA (2007b) indicates an average increase of 2.58 times in the degradation rate of pesticides when temperature is increased by 10 °C.

Triasulfuron dissipation increased in all the soils, but herbicide mobility in the soil profile also increased in S + GC2 from one month to more than five months of treatment. Residual concentrations of herbicide determined along S + GC2 profile could remain due to adsorption of herbicide by soil. In fact, significant correlations between the residual concentrations in the soil profile and the OC content were observed in this soil. Correlation coefficients (r) varied between 0.973 and 0.984 ($p < 0.01$) for the corresponding sampling times between 15 days and five months.

The DOC from the amended soil S + GC2 could help to promote the mobility of the herbicide in this soil after the second application showing it the persistence of this DOC in the soil profile and its impact on the leaching of triasulfuron over time. As already indicated, the DOC could be mobilized in the soil profile, and adsorb the mobilized triasulfuron, which would be consistent with the significant correlation found between the residual concentrations in S + GC2 and the DOC content in this soil from the second application (r ranged between 0.878 and 0.998, $p < 0.05$). The amount of additional irrigation applied after five months of herbicide application and the light rainfall did not have any relevant effects on the amount of herbicide leached. The herbicide was found to persist five months after its repeated application in the amended soil profile, although it decreased compared to that obtained after the first application.

4.3. Prosulfocarb mobility: effect of organic amendment and irrigation

Prosulfocarb is a non-ionic compound with low solubility in water, but its presence was detected in the soil profile after 13 days of treatment, especially in S and S + GC1. Simultaneously, prosulfocarb recorded a dissipation close to 50% in S and in S + GC1, while only a dissipation close to 10% was observed in S + GC2 in the same period.

After one month of treatment, the dissipation percentage of the compound increased in the unamended and amended soils, and after two months of treatment, the percentage of herbicide detected was < 3% in the amended soils, and it was not detected in the unamended soil. The highest prosulfocarb retention recorded in the 0–10 cm layer could be related to the soils' adsorption coefficients. The Kd adsorption coefficients were similar for S (21.6 ± 5.55 mL g^{-1}) and S + GC1 (24.7 ± 7.62 mL g^{-1}), but this coefficient increased significantly for S + GC2 (57.1 ± 2.09 mL g^{-1}) (Table S2 in Supplementary material) in agreement with the higher residual concentrations found in S + GC2. Prosulfocarb is adsorbed by S + GC2 to a greater extent than by S + GC1 due to the latter's higher OC content (Table 1). A significant correlation coefficient was found between residual concentrations and OC content in the soil profile for S + GC2 ($r \geq 0.976$, $p < 0.01$), S + GC1 ($r \geq 0.923$, $p < 0.05$) and S ($r \geq 0.813$, $p < 0.1$), despite the significant decrease in OC content in the soil profile. According to this, a significant correlation has been reported between the adsorption constants of prosulfocarb and soil OC contents (Marín-Benito et al., 2018; Nègre et al., 2006). Similar effects were observed for other carbamate compounds in unamended or amended soils (Berglöf et al., 2002; Marín-Benito et al., 2012).

The dissipation percentage of prosulfocarb was very high over the experimental period, especially in S and S + GC1. The dissipation in the topsoil layer was more significant than its mobility, as only herbicide traces were detected throughout the soil profile. Prosulfocarb is not very soluble in water, but when adsorbed by soil particles it may be transported out of the soil profile in S and S + GC1 by rainwater or by the additional irrigation applied, especially if random soil cracks occur, as indicated for triasulfuron. However, it should be noted that other dissipation processes could also be responsible for the high dissipation percentage of prosulfocarb, such as the microbiological degradation of the compound or volatilization. Prosulfocarb is considered as slightly volatile (PPDB, 2018) but it has been reported that considerable amounts of herbicide were lost from the soil in volatility experiments (EFSA, 2007a). Recently volatility of prosulfocarb from soils was also reported (Braun et al., 2017) as indicated for other thiocarbamates.

In S + GC2, the addition of the organic amendment might also have reduced the mobility of prosulfocarb by increasing the water holding-capacity of the 20-cm topsoil layers, which will be responsible for decreasing the water percolation, as indicated for triasulfuron. This circumstance and the greater adsorption of prosulfocarb by this soil could decrease its dissipation rate, although the prosulfocarb adsorbed by S + GC2 might also be affected by the processes indicated above.

4.4. *Prosulfocarb mobility: effect of repeated doses*

Similar to triasulfuron, the dissipation of prosulfocarb after repeated application was more rapid than after the first application. The volatilization of the herbicide could explain its high dissipation after 15 days of its application, as it could be favoured by the higher air temperature recorded at this time. On the other hand, the repeated prosulfocarb application could induce a greater soil microbial activity as it was indicated by Rouchaud et al. (1997) and this enhanced soil biodegradation could be favoured by the higher temperature recorded than in the first application. The residual concentrations increased in the order S < S + GC1 < S + GC2, following the same pattern as in the first application of the herbicide (Fig. 4). Simultaneously, the amounts recorded in the soil profile indicated the presence of prosulfocarb in all the soils, reaching up to 40–50 cm depth, especially in the case of S, despite the low amount of water received during this second application.

The residual concentrations remaining in the topsoil indicated that dissipation decreased slowly over time, without the complete disappearance of the compound in all the soils after the repeated application. The adsorption of herbicide by the soils could delay its dissipation. It should be noted that mobility in the soil profile was detected in all the soils, mainly after one month of the application of prosulfocarb, although the amounts retained decreased throughout the soil profile.

The decrease in the dissipation of the compound could be due to the low amount of water present in the soil during the five months following the second application. This circumstance could lower the mobility of the herbicide to deeper soil layers, avoid the presence of the herbicide in solution to be degraded, and/or generate a non-optimum scenario for its degradation by microorganisms. In fact, the influence of more irrigation (≈60 mm) than that supplied by rainfall (45 mm) after the second application could favour herbicide dissipation, as observed for all the soil treatments at different times.

By contrast, the effect of the DOC of S + GC2 could favour herbicide mobility in these amended soils after the second application. This would be consistent with the significant correlation found between the residual concentrations and the soil DOC content for each time (r ranging between 0.986 and 0.993, $p < 0.01$).

5. Conclusions

The results obtained confirm the mobility of triasulfuron and prosulfocarb up to 50 cm in the soil profile in unamended and amended soils with different doses of GC immediately after their application. The influence of the soil treatment (amendment) or environmental conditions (additional irrigation) was noted for both herbicides over time and it was recorded for the first and the second application of herbicides. The persistence in S + GC2 was higher than in S or S + GC1 and was explained by this soil's high capacity for adsorbing herbicides due to its OC content but prosulfocarb dissipated more quickly than triasulfuron. The leaching of both herbicides was affected by irrigation in all the soils although other factors, such as the DOC or water storage capacity may be significant in S + GC2. Both herbicides dissipated more rapidly after the second application than after the first application in all experimental conditions of soil, but residues of both herbicides were found in amended soils after five months of application. An accelerated degradation due to microbial adaptation leading to faster metabolism (triasulfuron and prosulfocarb) or an enhanced volatilization (prosulfocarb) could explain the increased dissipation after second application also enhanced by the increased temperature at this time. The interest of these results is that herbicides leaching from the soil surface may contaminate groundwater, and the amounts retained in the soil profile could exceed the recorded threshold for the sensitivities of susceptible species. Accordingly, establishing new agricultural practices involving organic residues requires the prior evaluation of their characteristics (OC and DOC) and the structural changes in soil aggregate that their application may imply in order to improve soil fertility without an environmental impact.

Conflicts of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jenvman.2018.07.045.

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Material Suplementario

SUPPLEMENTARY MATERIAL

RECYCLING ORGANIC RESIDUES IN SOILS AS AMENDMENTS: EFFECT ON THE MOBILITY OF TWO HERBICIDES UNDER DIFFERENT MANAGEMENT PRACTICES

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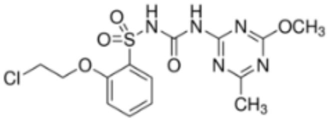
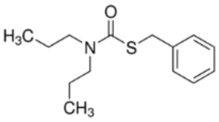
Table S1

Characteristics of herbicides (PPDB, 2018).

	Triasulfuron	Prosulfocarb
Chemical name (IUPAC)	1-[2-(2-chloroethoxy) phenylsulfonyl]-3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl) urea	*S*-benzyl dipropyl(thiocarbamate)
Chemical structure		
Water solubility (mg L^{-1})	815 (20°C, pH 7)	13.0 (20°C, pH 6.1)
log Kow	-0.59 (20°C, pH 7)	4.48 (20°C, pH 7)
DT_{50} (laboratory) (days)	59.1	12.4
DT_{50} (field) (days)	38.5	9.8
pKa	4.64 (at 25°C)	-
GUS index	5.12	0.84

PPDB, 2018. Pesticide Properties Data Base. University of Hertfordshire. http://sitem.herts.ac.uk/aeru/ppdb/en/index.htm.

Table S2

Adsorption coefficients (K_d) of triasulfuron and prosulfocarb by soil unamended and amended with green compost (GC) from experimental depths of soil profile.

Adsorption coefficient	Soil depth (cm)				
K_d (mL g^{-1})	0-10	10-20	20-30	30-40	40-50
Triasulfuron (Auros Plus®)	0.31±0.01[a]	0.30±0.04	0.28±0.04	0.32±0.04	0.29±0.01
	0.38±0.09[b]	-	-	-	-
	0.67±0.04[c]	-	-	-	-
Prosulfocarb (Auros Plus®)	21.6±5.55[a]	13.3±0.27	9.56±0.59	6.23±0.28	2.73±0.39
	30.7±7.62[b]	-	-	-	-
	57.1±2.09[c]				

[a]Unamended soil, [b]Soil amended with GC1, [c]Soil amended with GC2

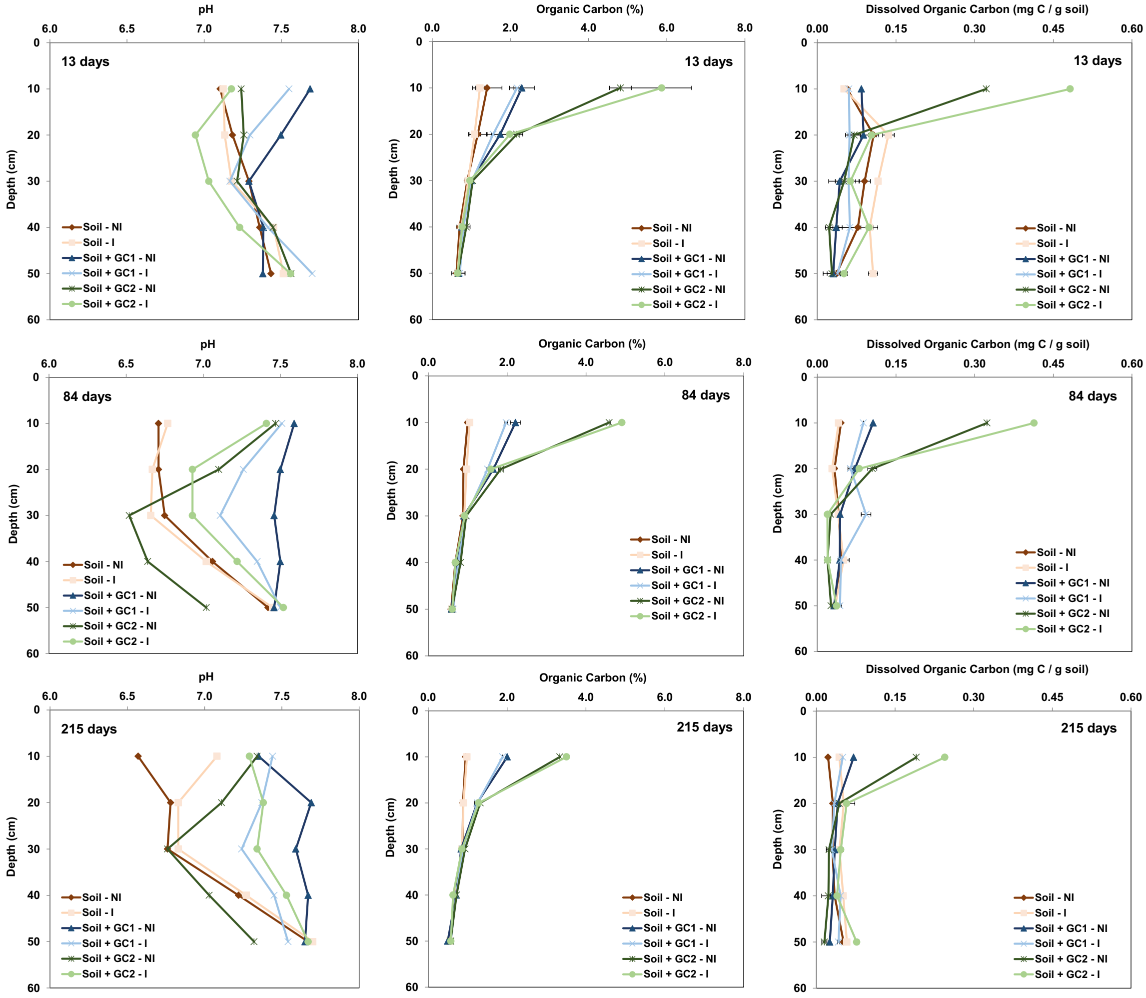


Figure S1.

Mean values of pH, organic carbon (OC) and dissolved organic carbon (DOC) for each 10-cm layer in soils unamended and amended with green compost (GC1 and GC2) under different irrigation conditions (non-irrigation (NI) and irrigation (I)) at different sampling times after the herbicides´ application. Times 84 and 215 days correspond to 15 and 147 days after the second application of herbicides. Error bars represent the standard deviation of mean values of plots treated (n=3).

CAPÍTULO 4

Resumen

CAPÍTULO 4: **Influence of different agricultural management practices on soil microbial community over dissipation time of two herbicides**

RESUMEN

En la actualidad se generan residuos orgánicos de diferente origen en grandes cantidades que son aplicados como enmiendas al suelo para mejorar su fertilidad. Sin embargo, la aplicación combinada de herbicidas y residuos orgánicos puede modificar el comportamiento fisicoquímico de los herbicidas en el suelo, lo cual puede tener un gran impacto en la diversidad y actividad de las comunidades microbianas del suelo.

En este trabajo se ha estudiado la influencia de diferentes prácticas agrícolas sobre la cinética de disipación de los herbicidas triasulfuron y prosulfocarb aplicados como una formulación comercial conjunta, Auros Plus®, en un suelo sin enmendar y enmendado con compost vegetal (GC) en condiciones de campo, así como los cambios que producen en la estructura de las comunidades microbianas durante el tiempo de disipación de los herbicidas.

Se estudiaron diferentes factores tales como la dosis de enmienda aplicada (~ 12 t C ha^{-1}, GC1 y 40 t C ha^{-1}, GC2), las condiciones de riego (sin riego y con riego adicional de 2.8 mm por semana) y el efecto de la aplicación repetida de los herbicidas. La dosis de herbicidas aplicada fue de 250 g i.a. ha^{-1} y 11.25 kg i.a. ha^{-1} para el triasulfuron y el prosulfocarb, respectivamente (2.5 veces la dosis recomendada) y la misma dosis se aplicó de nuevo cuando se alcanzó aproximadamente el 50% de disipación (68 días).

La velocidad de disipación del trisulfuron fue más lenta que la del prosulfocarb en el suelo y aumentó después de la segunda aplicación. Los valores de vida media (DT_{50}) fueron entre 1.3 y 3 veces menores que en la primera aplicación, mientras que los valores de DT_{50} para prosulfocarb no se modificaron después de la segunda aplicación. Los valores de DT_{50} de ambos herbicidas fueron más elevados en el suelo con el mayor porcentaje de GC debido a un aporte de carbono orgánico más elevado. La aplicación de riego adicional disminuyó los valores de DT_{50} de prosulfocarb pero no los de triasulfuron a pesar de su mayor solubilidad en agua.

La población microbiana total se comportó de manera similar con respecto a la presencia de los herbicidas en los suelos con y sin riego. Hubo un descenso de dicha población con la disipación de los herbicidas. La aplicación de los herbicidas no tuvo ningún efecto significativo en la población relativa de bacterias Gram negativas y Gram

positivas durante el experimento, pero la abundancia relativa de *Actinobacterias* aumentó en todos los suelos tratados. Al final del experimento (215 días), el efecto negativo de los herbicidas sobre la población de hongos fue significativo ($p<0.05$) en todos los tratamientos. Estos cambios microbiológicos fueron detectados en suelos con y sin riego adicional, y fueron más evidentes después de la segunda aplicación de los herbicidas. Los cambios microbiológicos de los suelos durante la segunda aplicación pueden ser los responsables del cambio en la velocidad de disipación de los herbicidas, que fue más rápida cuando la población de *Actinobacterias* se vio favorecida.

Este estudio contribuye de forma útil a la evaluación del efecto de la aplicación repetida de herbicidas a largo plazo bajo diferentes prácticas agrícolas de manejo del suelo y sus comunidades microbianas.

Artículo

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Influence of different agricultural management practices on soil microbial community over dissipation time of two herbicides

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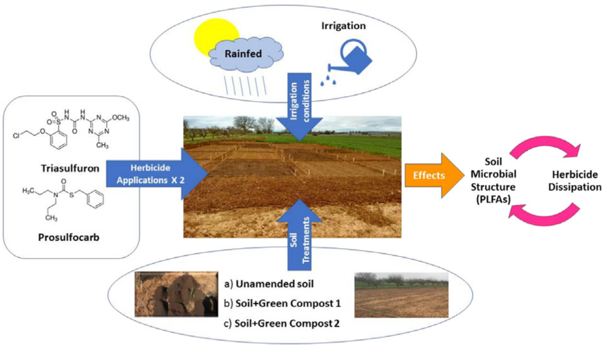
HIGHLIGHTS

- Agricultural practices affected herbicide dissipation and soil microbial structure.
- Repeated applications of herbicides accelerated the degradation of triasulfuron but not prosulfocarb.
- Higher content of organic matter resulted in increased herbicide residues in soils.
- Herbicides promoted the relative abundance of *Actinobacteria* and reduced fungi.
- The changes on soil microbiology produced by herbicides modify their degradation rates.

GRAPHICAL ABSTRACT



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ABSTRACT

Soil microbiology could be affected by the presence of pesticide residues during intensive farming, potentially threatening the soil environment. The aim here was to assess the dissipation of the herbicides triasulfuron and prosulfocarb, applied as a combined commercial formulation, and the changes in soil microbial communities (through the profile of phospholipid fatty acids (PLFAs) extracted from the soil) during the dissipation time of the herbicides under field conditions. The dissipation of herbicides and the soil microbial structure were assessed under different agricultural practices, such as the repeated application of herbicides (twice), in unamended and amended soils with two organic amendments derived from green compost (GC1 and GC2) and with non-irrigation and irrigation regimes. The results obtained indicate slower dissipation for triasulfuron than for prosulfocarb. The 50% dissipation time (DT_{50}) decreased under all conditions for the second application of triasulfuron, although not for prosulfocarb. The DT_{50} values for both herbicides increased in the GC2 amended soil with the highest organic carbon (OC) content. The DT_{50} values decreased for prosulfocarb with irrigation, but not for triasulfuron, despite its higher water solubility. The herbicides did not have any significant effects on the relative population of Gram-negative and Gram-positive bacteria during the assay, but the relative abundance of *Actinobacteria* increased in all the soils with herbicides. At the end of the assay (215 days), the negative effects of herbicides on fungi abundance were significant ($p < 0.05$) for all the treatments. These microbiological changes were detected in non-irrigated and irrigated soils, and were more noticeable after the second application of herbicides. *Actinobacteria* could be responsible for the modification of herbicide degradation rates, which tend

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to be faster after the second application. This study makes a useful contribution to the evaluation of the soil environment and microbiological risks due to the long-term repeated application of herbicides under different agricultural management practices.

1. Introduction

The structure of soil microbial communities and the changes produced in them by different environmental impacts is of great interest nowadays (Barra Caracciolo et al. 2015). Microbial activity is an accurate indicator of soil quality because soil microorganisms play a key role in organic matter (OM) decomposition and in the biogeochemical cycles that affect soil fertility (Pascual et al. 2000; García-Orenes et al. 2013). This microbial activity could be affected by the presence of pesticide residues in soil that pose a potential risk to soil ecology (Cycoń et al. 2013; Fang et al. 2018). Modern intensive farming involves the application of large quantities of pesticides during the crop growth period (Nyamwasa et al. 2018). In fact, the residues of herbicides, insecticides and fungicides have been detected in soils in agricultural areas across different countries in a broad range of concentrations (Li et al. 2014; Pose-Juan et al. 2015) which could modify soil microbial biodiversity. Residues of these agrochemicals depend on their dissipation in soils, being modified by different environmental factors (soil type, soil OM, weather, temperature, irrigation), pesticide formulation (individual or combined compounds), and application method (single or repeated application) (Arias-Estévez et al. 2008). This widespread use of pesticides could therefore lead to a potential decrease in soil microbial biodiversity, with a negative impact on crop yields (Baxter and Cummings 2008), which could be increased by the widespread loss of soil OM detected in recent years (Pascual et al. 2000).

A common practice used in agriculture to increase soil OM content involves the application of organic residues as soil amendments, with the aim being to improve soil fertility and stability, as well as stimulate microbial growth (Bastida et al. 2015). Organic residues of different origins (urban, agricultural or industrial) are generated in large quantities, and the improvement in soil properties due to their OM content has been well documented (Aranda et al. 2015; Bastida et al. 2015). However the combined application of pesticides and organic residues modifies the physicochemical behaviour of pesticides applied to soils, mainly through their adsorption-desorption (Marín-Benito et al. 2013, 2014). Changes in mobility or the formation of bound residues could occur depending on OM composition, which has implications regarding their bioavailability and total dissipation and consequences for overall soil microbial activity.

Studies on pesticide dissipation and its effects on soil microorganisms have been reported in unamended and amended soils. In general, these studies focus on the effect of a single compound with single application (Cycoń et al. 2013; Álvarez-Martín et al. 2016; Pose-Juan et al. 2017; Singh et al., 2018). They have been carried out in laboratory conditions, while few results have been reported from field experiments with more realistic environmental conditions (Herrero-Hernández et al. 2015). However, studies on the effects that combined application of pesticides have on their dissipation (Vischetti et al. 2008; Fang et al. 2018) and/or the effects of the repeated application of pesticides on soil microbial communities are scarcer in the literature (Baxter and Cummings 2008; Tortella et al. 2013; Fang et al. 2015; Wang et al. 2015). Soil microbial abundance and structure were evaluated through different approaches, and the part soil microorganisms play in the enhanced dissipation of pesticides after repeated applications has been reported. Nevertheless, these investigations have been scarcely assessed under field conditions using combined commercial formulations of pesticides, and most of these studies have been carried out in unamended soils (Kaur and Bhullar 2017; Kaur et al. 2017).

Triasulfuron (2-(2-chloroethoxy)-N-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) amino] carbonyl] benzene sulfonamide) is a mobile herbicide in soil due to its high water solubility and low hydrophobicity (EFSA 2015), while prosulfocarb (S-(phenylmethyl) dipropylcarbamothioate) is a hydrophobic herbicide with high adsorption, low mobility and a moderate persistence in soil (EFSA 2007a). These herbicides of the sulfonylurea and thiocarbamate group, respectively, are used on pre- and post-emergence in winter cereals (wheat, barley) and other crops (PPDB 2018). No study has been made of the combined long-term effect that triasulfuron and prosulfocarb have on their dissipation rates and soil microbial biomass and structure, although they are repeatedly applied to a broad range of crops. Compounds of the chemical groups sulfonamide and thiocarbamate, such as triasulfuron and prosulfocarb, are usually recommended in cereals for individual or joint use for controlling weeds in rainfed and irrigated cereal crops (Cirujeda and Taberner 2010; Bajya et al. 2015).

Relevant changes in soil microbial abundance, activity and structure have already been reported by the authors in a previous paper (García-Delgado et al. 2018) when triasulfuron and prosulfocarb were applied as a combined formulation in unamended and amended soils with green compost (GC) under field conditions. GC is the biodegradable organic residue from pruning in urban gardens and parks with an OM content higher than 15% (BOE 2013). The results were obtained for a single application of herbicides after the short-term dissipation of herbicides. Little is known about the other factors that could influence the dissipation of herbicides in soils and changes in the soil microbial structure under field conditions. Repeated herbicide application, different soil OC content from organic amendments, and/or irrigation may modify herbicide dissipation. The study of these factors would increase our knowledge on the effect herbicides have on soil microbial communities.

The aim here was therefore to assess the changes in soil microbial communities during the dissipation of two herbicides continuously applied under different agronomical practices. A combined commercial formulation of triasulfuron and prosulfocarb (Aurus Plus®) was applied twice to an unamended soil and one amended with two organic amendments derived from green compost (GC1 and GC2) and with non-irrigation and irrigation regimes. The effect of these factors on the dissipation of herbicides (DT_{50}) and on the soil microbial structure was studied under field conditions. The study makes a useful contribution to the evaluation of environmental and microbiological risks due to the combined long-term application of herbicides under different management practices in agriculture.

2. Materials and methods

2.1. Herbicides

The commercial formulation of triasulfuron (TSF) (2-(2-chloroethoxy)-N-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) amino] carbonyl] benzene sulfonamide) (20% p/p) and prosulfocarb (PSC) (S-(phenylmethyl) dipropylcarbamothioate) (80% p/v) (Auros Plus®, Syngenta Agro S.A., Madrid, Spain) was used in the field study. Analytical standards of both herbicides (purity > 98.9%) were supplied by Sigma Aldrich Química S.A. (Madrid, Spain). Water solubility is 815 and 13.0 mg L^{-1} and log K_{ow} is −0.59 and 4.48 for triasulfuron and prosulfocarb, respectively (PPDB 2018).

2.2. Organic residues

Two green composts formed by composted vegetal residues were used. They were supplied by the local authority (GC1) and by the nursery "El Arca" (GC2) from Salamanca (Spain). Their main physicochemical characteristics on a dry weight basis were determined by standard methods (Sparks et al., 1996) and are as follows: pH 7.33 and 7.58, organic carbon (OC) content 9.80% and 24.1%, dissolved organic carbon (DOC) content 0.353% and 0.700%, total N 1.04% and 1.10%, C/N ratio 9.42 and 21.9, and ash percentage 74.5% and 53.0% for GC1 and GC2, respectively.

2.3. Experimental set-up

A field experiment was conducted in an agricultural soil (sandy clay loam soil, *Typic Haploxerept*) in the Muñovela experimental farm belonging to IRNASA-CSIC (Salamanca, Spain). An experimental layout of randomized complete blocks was designed in February 2016, with six treatments and three replicates per treatment (18 plots of 9 m^2) corresponding to unamended soil (6 plots, S) and soil amended with GC1 (6 plots, S + GC1) or GC2 (6 plots, S + GC2) at the rate of 120 and 180 t ha^{-1} on a dry weight basis, respectively. For each soil treatment, three plots received only natural rainfall, while other three plots received weekly 2.8 mm (I). In March 2016, the commercial formulation of triasulfuron and prosulfocarb (Auros Plus®) was applied to 18 experimental plots at doses of 250 g a.i. ha^{-1} and 11.25 kg a.i. ha^{-1}, respectively, corresponding to 2.5 times the maximum agronomic dose for both herbicides recommended for heavy soils with a greater adsorption capacity. The increase in the soil's capacity for adsorbing the herbicides after an organic amendment supports the use of doses higher than those recommended to maintain the efficacy of the compounds. Once the herbicide half-lives (DT_{50}) were achieved in all plots (after 68 days), the herbicides were applied again at the same doses in May 2016. A check was made prior to the application of the herbicides to ensure that no amounts of these compounds were detectable in the soil samples. This was as expected, because the plots had no history of triasulfuron and prosulfocarb application in the previous five years. Additionally, eighteen control plots (six by soil treatment) did not receive herbicide application, but nine of them received irrigation.

Weather conditions were recorded throughout the experiment (215 days) by a meteorological station located on site (Fig. S1 in Supplementary material). Air temperature ranged from 1.7 °C to 14.6 °C (mean air temperature 8.3 °C) and from 10.5 °C to 26.3 °C (mean air temperature 19.1 °C) during the first (0–68 days) and the second (69–215 days) period of the application, respectively. Cumulative precipitation and additional irrigation were 139.2 mm and 11.2 mm during the first application, and 46.6 mm and 58.8 mm during the second application of herbicides, respectively. Total cumulative precipitation and additional irrigation were 185.8 mm and 70 mm, respectively.

Table 1
Characteristics of unamended and GC amended soils, non-irrigated or irrigated (I) after the first and the second application of herbicides in the field plots (sampling times corresponding to 13 days and 84 days after the beginning of the experiment).

	pH	OC[a] (%)	DOC[b] (mg kg^{-1})	N (%)	C/N
Soil	7.10–6.71	1.41–0.98	0.057–0.045	0.12–0.08	11.8–12.2
Soil-I	7.12–6.77	1.22–1.05	0.051–0.041	0.11–0.10	11.1–10.5
Soil + GC1	7.69–7.59	2.29–2.09	0.084–0.106	0.19–0.21	12.1–9.95
Soil + GC1-I	7.55–7.51	2.17–2.07	0.060–0.088	0.20–0.20	10.8–10.4
Soil + GC2	7.24–7.47	4.81–3.75	0.322–0.324	0.43–0.33	11.2–11.4
Soil + GC2-I	7.18–7.41	5.87–4.32	0.482–0.413	0.46–0.37	12.8–11.7

[a] Organic carbon.
[b] Dissolved organic carbon.

2.4. Soil sampling and herbicide extraction and analysis

Surface soil samples from 0 to 10 cm were collected to determine herbicide dissipation at different times between 0 and 68 days (first herbicides' application) and between 69 and 215 days (second herbicides´ application) and to determine soil microbial structure at 0, 28, 69, 97, 124 and 215 days. Soil samples were collected, processed and characterized following the methods described by Marín-Benito et al. (2018a) and García-Delgado et al. (2018) (complete information is included in Supplementary material). Soil characteristics are included in Table 1. Soil samples were sieved (<2 mm) and moisture content of the bulk sample was determined. Duplicate subsamples of moist soil (6 g) were extracted with methanol (12 mL) by shaking and sonication. The determination of the herbicides in the soil extracts was performed by HPLC-MS (Waters Assoc., Milford, MA, USA). The molecular ions [m/z] 402.8 (triasulfuron) and 252.4 (prosulfocarb) were monitored and the retention times were 6.1 min and 14.1 min, respectively. A detailed description of the herbicide extraction and analytical methods is included in Supplementary material.

2.5. Soil microbial community by PLFA analysis

The soil microbial community composition of the soil samples was determined using phospholipid fatty acid (PLFA) analysis, as described in Frostegård et al. (1993). Lyophilized soils samples were extracted with a one-phase chloroform-methanol-phosphate buffer solvent by sonication. Extracts were purified by SPE and polar lipids were transesterified with methanol-KOH. Finally, hexane extracts containing the resultant fatty acid methyl esters were analyzed by gas chromatography. Quantification was performed using an Agilent 7890 gas chromatograph (Agilent Technologies, Wilmington, DE, USA) equipped with a 25-m Ultra 2 (5% phenyl)-methylpolysiloxane column (J&W Scientific, Folsom, CA, USA) and a flame ionization detector. PLFAs were identified using bacterial fatty acid standards and software from the Microbial Identification System (Microbial ID, Inc., Newark, DE, USA). Specific PLFAs (Zelles, 1999) were used as biomarkers to quantify the relative abundances of Gram negative (monounsaturated fatty acids and cyclopropyl 17:0 and 19:0) and Gram positive (iso and anteiso saturated branched fatty acids) bacteria, *Actinobacteria* (10-methyl fatty acids) and fungi (18:2 ω6 cis).

2.6. Data analysis

The dissipation kinetics for triasulfuron and prosulfocarb were fitted to a single first-order (SFO) or first-order multicompartment (FOMC) models and values for the time to 50% dissipation (DT_{50}) were estimated using the Excel Solver add-in package (FOCUS, 2006). More details about the fitting of the dissipation kinetics are included in Supplementary material.

Analysis of variance (ANOVA) was used to evaluate the effects of the different treatments (green compost application, repeated herbicide application and irrigation) on herbicide dissipation. Standard deviation (SD) was used to indicate variability among replicates. Fisher's least significant difference (LSD) method, at a confidence level of 95%, was determined with IBM SPSS Statistics v24 software package (SPSS Inc. Chicago, USA).

Data of PLFAs were submitted for the analysis of variance (ANOVA) by previous Levene variance homogeneity test to determine significant differences between treatments at each sampling time. Means were compared by either Tukey or Games–Howell post hoc test based on whether or not variance homogeneity was met, respectively ($p <$ 0.05). Pearson correlation coefficients between the remaining concentration and percentages of herbicides, and microbial biomass and structure were determined to elucidate how variables are related to each other. ANOVA and correlation analyses were carried out using the IBM SPSS Statistics v24 software package (SPSS Inc. Chicago, USA). Principal component analysis (PCA) was performed, with PAST v3.15 software (Hammer et al. 2001), to determine the most meaningful variables and the global impact of the herbicides and soil treatments on soil microbial community. In addition to PCA, PERMANOVA analysis was performed to determine the significance of herbicides application, sampling time, soil treatments and their interactions.

3. Results and discussion

3.1. Herbicide dissipation after repeated application in soils, soil amendment, and irrigation conditions

The dissipation kinetics of herbicides after the first and second application fit the SFO model for most of the treatments, and only in four plots the dissipation kinetics provide a better fit for the FOMC model (Figs. S2 and S3 in Supplementary material). Other authors also report that the SFO equation is the model that best fits triasulfuron and prosulfocarb dissipation in the field (Rouchaud et al. 1997; Sarmah et al. 2000). The DT_{50} values were used to compare the dissipation rates of triasulfuron and prosulfocarb under the different conditions studied (herbicide type, repeated dose, soil amendment, and irrigation) (Tables 2 and 3). The dissipation curves show a continuous decrease in triasulfuron and prosulfocarb concentrations over time. It is faster for prosulfocarb than for triasulfuron for all the treatments studied, as indicated by the lower DT_{50} values. The faster dissipation of prosulfocarb could be due to processes of adsorption, biodegradation, mineralization, and/or volatilization, as reported previously (Marín-Benito et al., 2018b; Braun et al., 2017; EFSA, 2007a).

The DT_{50} values for both herbicides after the first application were consistent with those determined in a previous work, when they were applied as a single or combined formulation in unamended or GC-amended soils, albeit in a lower dose than in this study (Marín-Benito et al., 2018a). The repeated application of triasulfuron has an effect on its persistence, with higher dissipation rates, and DT_{50} values 1.3 to 3 times lower than after the first application (Table 2). These results indicate that the persistence of triasulfuron was lower after the second application. This is consistent with the remaining percentages of triasulfuron at 68 days after the first application, ranging between 14%–51%, and at 69 days after the second application (corresponding to 138 days after the first application), being between 3%–17% in the soils with the different treatments. Other authors have also reported accelerated dissipation after repeated pesticide applications due to the faster metabolism caused by enhanced biodegradation, which could lead to a reduction in pesticide efficacy in some cases (Baxter and Cummings 2008; Fang et al. 2018).

The DT_{50} values for prosulfocarb decreased by up to 1.3 and 1.6 times after its second application in irrigated S and S + GC2 soils, respectively, but the DT_{50} values were, in general, similar for all the other soil treatments (Table 3). However, the remaining percentages of prosulfocarb at 68 days after the first application were lower (0%–3%) than those at 69 days after the second application (corresponding to 138 days after the first application) (2%–21%). These results indicate that the amounts of prosulfocarb remaining after the second application decreased more slowly over time than after the first application, without fully dissipating. Rouchaud et al. (1997) have reported that repeated prosulfocarb application to a barley crop enhanced soil biodegradation. However, the decrease in prosulfocarb concentrations after the second application and over the course of 50% dissipation was slower than after the first application, and this resulted in a higher persistence of the herbicides in the soil at the end of the assay (215 days) (Fig. S3).

The DT_{50} values for the dissipation of triasulfuron and prosulfocarb after the two applications were higher in S + GC2 than in S or S + GC1. The DT_{50} values increased by 3.1–1.8 times and by 2.0–1.9 times compared to the unamended soil. These results are related to the higher OC content in S + GC2 than in S + GC1, which could help increase the persistence of these herbicides in the top soil, decreasing its leaching (Marín-Benito et al., 2018b). In fact, a significant and positive correlation ($R^2 = 0.828$, $p < 0.001$) was found between the DT_{50} values of triasulfuron and prosulfocarb and OC content. Furthermore, the adsorption of both herbicides by S + GC2 could occur with the possible

Table 2

Dissipation parameters and goodness of fit for triasulfuron after repeated application (1 and 2) in unamended or amended soils under non-irrigated or irrigated conditions calculated by fitting the SFO or FOMC models.

Sample plot	Non-irrigated soils				Irrigated soils			
	k (days^{-1})	DT_{50} (days)	χ^2	R^2	k (days^{-1})	DT_{50} (days)	χ^2	R^2
Soil								
A -1	0.023	30.3 ± 1.84	7.3	0.95	0.026	26.2 ± 0.89	12.9	0.89
-2	0.045	15.5 ± 0.46	8.6	0.97	0.039	17.6 ± 0.12	8.5	0.97
B -1	0.020	34.1 ± 0.05	7.7	0.94	0.030	23.3 ± 0.90	13.2	0.89
-2	0.046	15.0 ± 0.02	12.1	0.97	0.047	14.9 ± 0.15	9.6	0.97
C -1	0.026	27.2 ± 0.50	10.7	0.91	0.026	26.8 ± 0.95	10.4	0.92
-2	0.042	16.6 ± 0.71	10.7	0.97	0.043	16.3 ± 0.50	8.3	0.98
Mean -1		30.5 ± 3.46c				25.4 ± 1.87bc		
-2		15.7 ± 0.82a				16.3 ± 1.35a		
Soil+GC1								
A -1	0.023	30.0 ± 0.19	6.7	0.96	0.024	28.6 ± 2.18	14.9	0.84
-2	0.038	18.3 ± 0.28	12.5	0.96	0.033	20.7 ± 0.46	17.5	0.89
B -1	0.019	36.0 ± 2.32	12.6	0.83	0.023	30.1 ± 0.75	10.0	0.90
-2	0.031	22.5 ± 0.11	9.8	0.97	0.030	23.1 ± 0.73	7.5	0.97
C -1	0.029	24.1 ± 1.99	14.7	0.87	0.025	27.4 ± 0.56	13.5	0.87
-2	0.032	21.7 ± 0.01	9.1	0.98	0.034	20.5 ± 0.72	10.3	0.97
Mean -1		30.0 ± 5.95c				28.7 ± 1.35bc		
-2		20.8 ± 2.23ab				21.4 ± 1.45ab		
Soil+GC2								
A -1	0.341–8.818[a]	59.2 ± 3.61	5.8	0.93	0.008	91.1 ± 8.40	1.7	0.97
-2	0.030	23.0 ± 1.39	10.1	0.83	0.021	32.6 ± 1.71	6.3	0.97
B -1	0.008	90.5 ± 3.96	2.9	0.94	0.010	72.4 ± 0.44	4.6	0.92
-2	0.020	34.5 ± 8.94	9.7	0.86	0.033	21.0 ± 1.21	5.3	0.99
C -1	0.518–17.68[a]	55.1 ± 2.97	4.1	0.97	0.010	72.7 ± 9.85	4.9	0.86
-2	0.024	28.4 ± 0.32	8.7	0.97	0.028	25.0 ± 0.46	8.0	0.97
Mean -1		68.3 ± 17.6d				78.7 ± 10.7e		
-2		28.6 ± 5.75bc				26.2 ± 5.89bc		

[a] Dissipation parameters (α and β) calculated from fitting experimental data to the FOMC model. Different letters in DT_{50} values indicate significant differences among samples and treatments (LSD = 7.963, $p < 0.05$).

Table 3
Dissipation parameters and goodness of fit for prosulfocarb after repeated application (1 and 2) in unamended or amended soils under non-irrigated or irrigated conditions calculated by fitting the SFO or FOMC models.

Sample/plot	Non-irrigated soils				Irrigated soils			
	k (days^{-1})	DT_{50} (days)	χ^2	R^2	k (days^{-1})	DT_{50} (days)	χ^2	R^2
Soil								
A -1	0.085	8.2 ± 0.42	14.9	0.96	0.070	9.9 ± 0.99	12.1	0.96
-2	0.067	10.3 ± 0.02	13.7	0.97	0.106	6.5 ± 0.86	11.4	0.98
B -1	0.059	11.8 ± 0.34	14.2	0.94	0.076	9.1 ± 0.62	8.4	0.98
-2	0.060	11.6 ± 0.32	14.9	0.94	0.090	7.7 ± 0.03	14.9	0.97
C -1	0.080	8.6 ± 0.37	14.6	0.93	0.077	9.0 ± 0.71	13.4	0.96
-2	0.069	10.0 ± 0.41	14.4	0.95	0.097	7.2 ± 0.45	15.0	0.95
Mean -1		9.5 ± 1.97ab				9.3 ± 0.49ab		
-2		10.6 ± 0.85b				7.1 ± 0.60a		
Soil + GC1								
A -1	0.055	12.5 ± 0.43	13.9	0.96	0.064	10.8 ± 0.27	11.5	0.97
-2	0.051	13.5 ± 0.03	11.7	0.96	0.073	9.5 ± 0.24	14.2	0.94
B -1	0.060	11.5 ± 0.08	9.2	0.98	0.071	9.8 ± 0.48	5.1	0.99
-2	1.284–12.91[a]	9.2 ± 0.54	11.4	0.98	0.070	9.9 ± 0.19	14.9	0.87
C -1	0.077	9.1 ± 0.56	10.5	0.98	0.080	8.7 ± 0.13	9.3	0.99
-2	1.461–16.0[a]	9.7 ± 0.72	14.5	0.95	0.061	11.5 ± 0.47	13.7	0.97
Mean -1		11.0 ± 1.75b				9.8 ± 1.05ab		
-2		10.8 ± 2.35b				10.3 ± 1.06ab		
Soil+GC2								
A -1	0.033	21.0 ± 1.19	11.9	0.94	0.033	21.2 ± 0.06	14.7	0.93
-2	0.051	13.6 ± 0.02	14.9	0.94	0.063	11.2 ± 0.00	14.8	0.97
B -1	0.037	18.6 ± 0.34	9.7	0.97	0.039	17.6 ± 0.42	11.0	0.97
-2	0.046	15.0 ± 0.32	13.5	0.86	0.063	11.1 ± 0.73	15.0	0.95
C -1	0.036	19.1 ± 0.70	9.0	0.97	0.039	17.9 ± 0.41	9.4	0.97
-2	0.021	32.4 ± 0.97	13.3	0.88	0.055	12.6 ± 0.20	14.4	0.92
Mean -1		19.6 ± 1.27c				18.9 ± 2.00c		
-2		20.3 ± 10.5c				11.6 ± 0.84b		

[a] Dissipation parameters (α and β) calculated from fitting experimental data to the FOMC model. Different letters in DT_{50} values indicate significant differences among samples and treatments (LSD = 3.227, $p < 0.05$).

formation of bound residues and a potential decrease in bioavailability and biodegradation (Gennari et al. 2002; Said-Pullicino et al. 2004). The relationship between the adsorption and degradation of these herbicides has also been reported in previous works (Nègre et al., 2006; Said-Pullicino et al., 2004). A significant and positive correlation was also found here between the K_d determined for triasulfuron (S, 0.31 ± 0.01 mL g^{-1}; S + GC1, 0.38 ± 0.09 mL g^{-1} and S + GC2, 0.67 ± 0.03 mL g^{-1}) or for prosulfocarb (S, 21.6 ± 5.55 mL g^{-1}; S + GC1, 24.7 ± 7.62 mL g^{-1} and S + GC2, 57.1 ± 2.09 mL g^{-1}) (Marín-Benito et al., 2018b) and the DT_{50} values determined for the first and second dissipation kinetics ($R^2 = 0.665$, $p < 0.02$, triasulfuron) and ($R^2 = 0.860$, $p < 0.001$, prosulfocarb).

Additional irrigation did not significantly modify the dissipation rates of triasulfuron after the two applications in S—I or S + GC1-I, but it decreased in S + GC2-I after the first application. Similarly, Sarmah et al. (2000) have reported that the DT_{50} values of triasulfuron in an unamended soil under field conditions remained unchanged when the soil received 89 mm of irrigation compared to the non-irrigated soil. The DT_{50} values of prosulfocarb were similar in irrigated soils after the first herbicide application. However, the dissipation rates increased in S—I and to a greater extent in S + GC2-I after the second herbicide application. Irrigation could lead to a higher potential degradation and/or leaching of herbicides through the soil profile.

The additional evaluation of the influence of overall weather conditions on the dissipation of triasulfuron and prosulfocarb revealed the possible effect of temperature for explaining the accelerated degradation of triasulfuron after the second application. The average temperature differed during the two dissipation periods, increasing by 10.8 °C after the second herbicide application. This result is consistent with the increase in the degradation rate of 2.58 times when the temperature increases by 10 °C, as determined by the European Food Safety Authority (EFSA 2007b). Dinelli et al. (1998) have reported that temperature had an effect on the degradation rate of triasulfuron, and the DT_{50} value decreased three times when temperature increased from 10 °C to 20 °C. Stork (1995) has observed that triasulfuron degradation rates increased with soil temperature, but they were not affected by soil water content. Temperature had no effect for prosulfocarb after the second application of herbicide because faster dissipation occurred only in irrigated treatments of S—I and S + GC2-I and could be explained by other processes, as previously indicated.

3.2. Effect of herbicides residues, organic amendments and irrigation regimes on the soil microbial structure

The total microbial population behaved in a similar way towards herbicides in both irrigated and non-irrigated soils. The amounts of herbicide residues, in total (μg g^{-1}) or relative concentration (%), were positively related to the total microbial population (nmol/g) (Tables 4 and 5). It means a decrease in this population while the dissipation of herbicides occurs. The toxicity of pesticides towards soil microorganisms is well described in the literature, mainly in high doses (El Azhari et al., 2018; Fang et al., 2018; Franco-Andreu et al., 2016a; Kalia and Gosal, 2011; Wang et al., 2015). However, previous studies do not provide consistent results on the toxicity of triasulfuron in soil microbiology between laboratory and field scale. Lupwayi et al. (2004) and Pose-Juan et al. (2017) report that triasulfuron has no toxic effects on microbial biomass at field and laboratory scale, respectively. In contrast, Sofo et al. (2012) have reported toxic effects at laboratory scale for an agronomic dose or higher. A recent study at field scale using a lower dose of triasulfuron than in this work agreed with the latter, indicating a decrease in microbial biomass due to the toxic effects of triasulfuron, and more so a combination of triasulfuron and prosulfocarb in the unamended and GC amended soils (García-Delgado et al., 2018). Therefore, there is evidence of the toxicity of triasulfuron and prosulfocarb towards soil microbiota. Additionally, our results indicated that the bacteria/fungi ratio was negatively correlated with the herbicide residues,

Table 4

Pearson correlation coefficients of non-irrigated soils between population of Gram−, Gram+, *Actinobacteria*, fungi and their corresponding relative concentrations, total biomass, ratio Gram−/ total Gram+, ratio bacteria/fungi, remaining concentrations of triasulfuron (TSF) and prosulfocarb (PSC) and remaining percentage of TSF and PSC. Significant correlations were denoted by asterisks and blond font.

No irrigation	Gram− (nmol/g)	Gram+ (nmol/g)	*Actinobacteria* (nmol/g)	Fungi (nmol/g)	Biomass (nmol/g)	Gram−/Gram+	Bacteria/fungi	Gram− (%)	Gram+ (%)	*Actinobacteria* (%)	Fungi (%)	[TSF]	[PSC]	TSF (%)	PSC (%)
Gram− (nmol/g)	1														
Gram+ (nmol/g)	**0.960*****	1													
Actinobacteria (nmol/g)	**0.936*****	**0.952*****	1												
Fungi (nmol/g)	**0.576*****	**0.575*****	**0.506*****	1											
Biomass (nmol/g)	**0.986*****	**0.989*****	**0.966*****	**0.604*****	1										
Gram−/Gram+	**−0.313***	**−0.535*****	**−0.551*****	−0.257	**−0.455****	1									
Bacteria/fungi	−0.169	−0.149	−0.077	**−0.740*****	−0.177	−0.021	1								
Gram− (%)	**−0.288***	**−0.510*****	**−0.505*****	**−0.422****	**−0.435****	**0.970*****	0.168	1							
Gram+ (%)	**0.535*****	**0.733*****	**0.600*****	**0.301***	**0.634*****	**−0.789*****	0.020	**−0.765*****	1						
Actinobacteria (%)	**−0.318****	**−0.344***	−0.087	**−0.504*****	**−0.331***	−0.243	**−0.390****	−0.187	−0.256	1					
Fungi (%)	0.172	0.145	0.041	**0.669*****	0.158	0.067	**−0.952*****	−0.053	0.02	**−0.401****	1				
[TSF]	**0.652*****	**0.651*****	**0.609*****	**0.478*****	**0.659*****	−0.261	−0.247	0.266	**0.412****	**−0.330***	**0.283***	1			
[PSC]	**0.602*****	**0.597*****	**0.573*****	**0.368****	**0.605****	−0.220	−0.223	−0.212	**0.341***	−0.207	**0.301***	**0.811*****	1		
%TSF	**0.614*****	**0.624*****	**0.570*****	**0.535*****	**0.639*****	−0.196	**−0.362***	−0.217	**0.359****	**−0.378****	**0.406****	**0.895*****	**0.867*****	1	
%PSC	**0.627*****	**0.612*****	**0.574*****	**0.498*****	**0.628*****	−0.196	**−0.370***	−0.219	**0.337***	**−0.290***	**0.404****	**0.863*****	**0.919*****	**0.930*****	1

[*] $p < 0.050$.

[**] $p < 0.010$.

[***] $p < 0.001$.

Table 5

Pearson correlation coefficients of irrigated soils between population of Gram−, Gram+, *Actinobacteria*, fungi and their corresponding relative concentrations, total biomass, ratio Gram−/ total Gram+, ratio bacteria/fungi, remaining concentrations of triasulfuron (TSF) and prosulfocarb (PSC), and remaining percentage of TSF and PSC. Significant correlations were denoted by asterisks and blond font.

Irrigation	Gram− (nmol/g)	Gram+ (nmol/g)	*Actinobacteria* (nmol/g)	Fungi (nmol/g)	Biomass (nmol/g)	Gram−/Gram+	Bacteria/fungi	Gram− (%)	Gram+ (%)	*Actinobacteria* (%)	Fungi (%)	[TSF]	[PSC]	TSF (%)	PSC (%)
Gram− (nmol/g)	1														
Gram+ (nmol/g)	**0.937*****	1													
Actinobacteria (nmol/g)	**0.865*****	**0.963*****	1												
Fungi (nmol/g)	**0.763*****	**0.652*****	**0.520*****	1											
Biomass (nmol/g)	**0.977*****	**0.986*****	**0.939*****	**0.738*****	1										
Gram−/Gram+	0.019	**−0.311***	**−0.436****	0.163	−0.176	1									
Bacteria/fungi	**−0.420****	−0.230	−0.120	**−0.710*****	**−0.342***	**−0.481*****	1								
Gram− (%)	−0.088	**−0.413****	**−0.505*****	−0.071	**−0.291***	**0.963*****	**−0.302***	1							
Gram+ (%)	**0.305***	**0.595*****	**0.617*****	−0.011	**0.460****	**−0.854*****	**0.425****	**−0.844*****	1						
Actinobacteria (%)	**−0.620*****	**−0.400****	−0.183	**−0.705*****	**−0.497*****	**−0.652*****	**0.665*****	**−0.492*****	0.250	1					
Fungi (%)	**0.433****	0.273	0.134	**0.906*****	**0.380****	**0.447****	**−0.856*****	0.232	**−0.355****	**−0.723*****	1				
[TSF]	**0.782*****	**0.778*****	**0.701*****	**0.667*****	**0.795*****	−0.035	**−0.476*****	−0.151	0.247	**−0.491*****	**0.413****	1			
[PSC]	**0.670*****	**0.733*****	**0.731*****	**0.420****	**0.716****	−0.230	−0.248	**−0.293***	**0.373****	−0.211	0.188	**0.795*****	1		
%TSF	**0.775*****	**0.782*****	**0.750*****	**0.623*****	**0.797*****	−0.087	**−0.474*****	−0.198	0.262	**−0.403****	**0.390****	**0.904****	**0.903*****	1	
%PSC	**0.770*****	**0.811*****	**0.777*****	**0.601*****	**0.809*****	−0.183	**−0.360***	**−0.277***	**0.350****	**−0.346***	**0.316***	**0.875*****	**0.942*****	**0.952*****	1

[*] $p < 0.050$.

[**] $p < 0.010$.

[***] $p < 0.001$.

i.e. the soils had more bacteria and a lower population of fungi with respect to the initial situation. This effect has also been observed in soils fumigated with imazethapyr, herbicide with the same mechanisms of action than triasulfuron (Zhang et al., 2010).

The presence of triasulfuron and prosulfocarb reveal different effects on microbial structure in both irrigated and non-irrigated soils during the field assay. The most abundant microorganisms in the irrigated and non-irrigated soils were Gram-negative bacteria followed by Gram-positive bacteria, *Actinobacteria,* and finally fungi (Figs. 1 and 2). A different composition of the microbial structure could be expected due to the different irrigation regime and organic amendment management, as reported by Franco-Andreu et al. (2016a, 2016b) and Sun et al. (2017). These authors reported that non-irrigated soils have been related to a higher proportion of Gram-positive bacteria than in irrigated

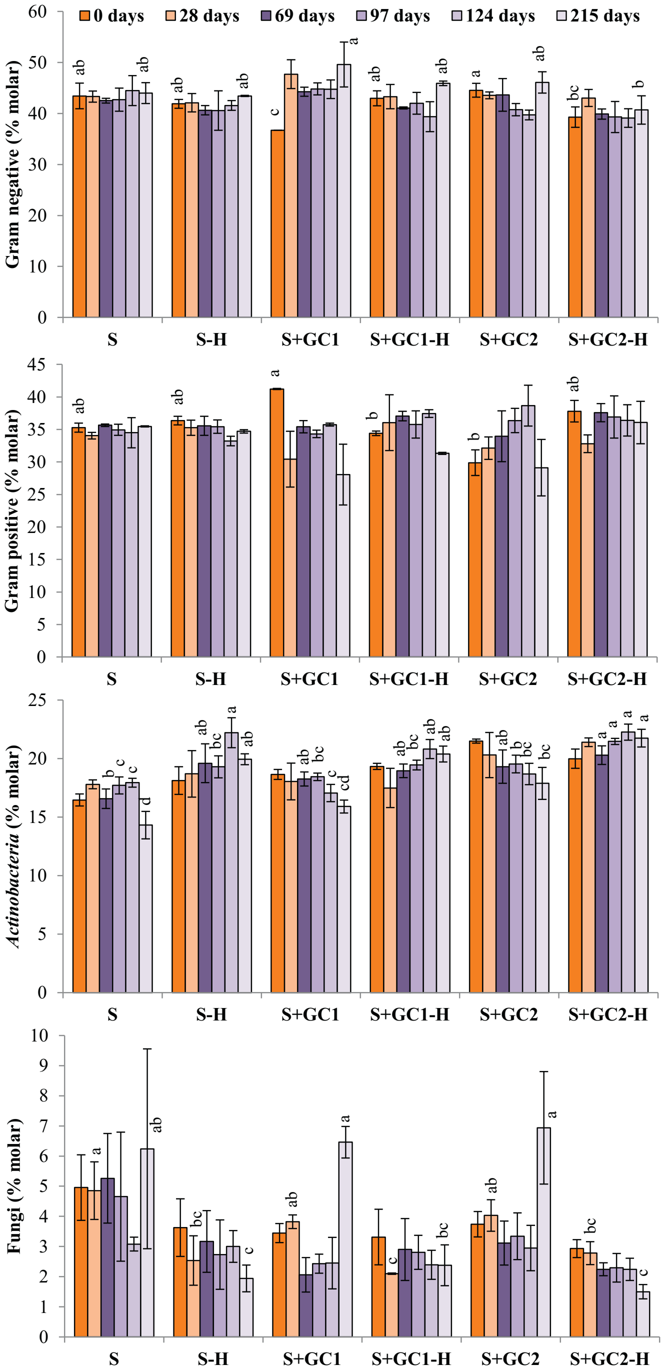

Fig. 1. Relative abundance (%mol) of PLFAs specifically diagnostics of Gram− and Gram+ bacteria, *Actinobacteria* and fungi in the non-irrigated soils. Vertical bars represent the standard deviation of three replicates. Different letters indicate significant differences among treatments at the same sampling time (Tukey post hoc test, $p < 0.05$). S: unamended soil; S + GC1: amended soil with green compost 1; S + GC2: amended soil with green compost 2; H: herbicides application. The first application of herbicides is denoted by brown colour and the second application by purple colour.

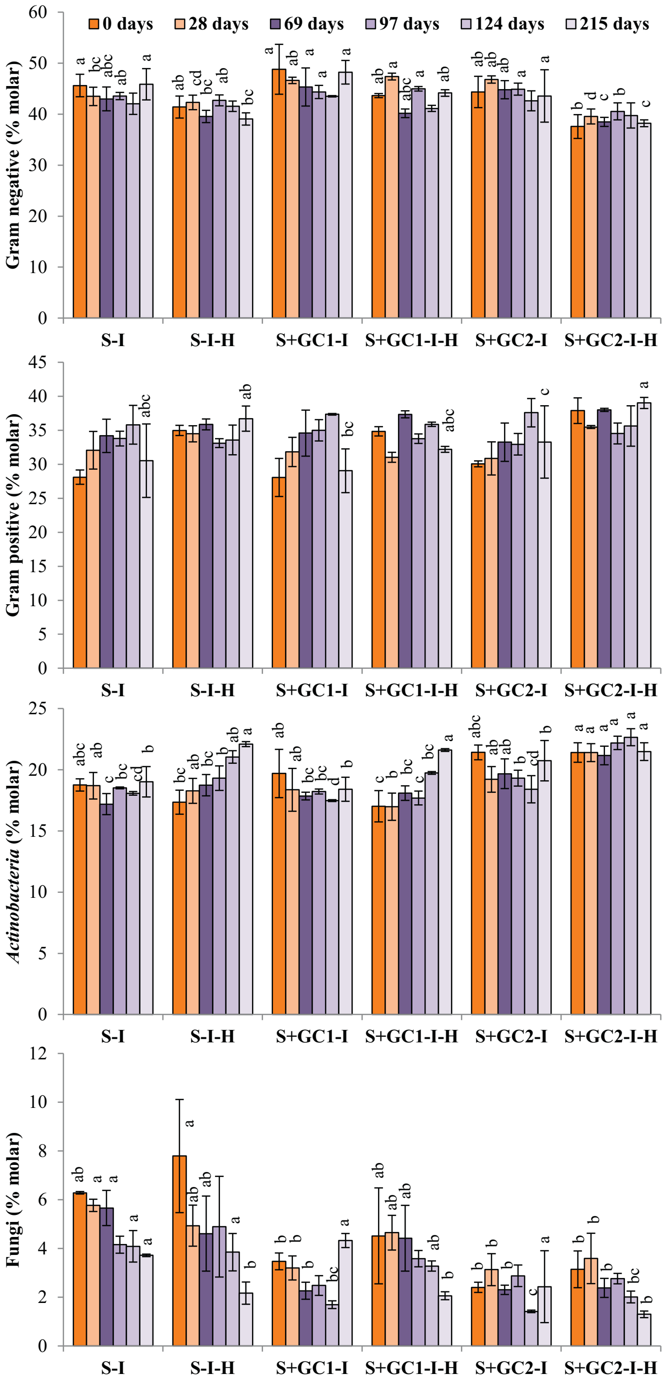

Fig. 2. Relative abundance (%mol) of PLFAs specifically diagnostics of Gram− and Gram+ bacteria, *Actinobacteria* and fungi in the irrigated soils. Vertical bars represent the standard deviation of three replicates. Different letters indicate significant differences among treatments at the same sampling time (Tukey post hoc test, $p < 0.05$). S: unamended soil; S + GC1: amended soil with green compost 1; S + GC2: amended soil with green compost 2; H: herbicides application; I: irrigation. The first application of herbicides is denoted by brown colour and the second application by purple colour.

soils because of the higher rigidity of cell walls compared to Gram-negative bacteria. However, the application of organic amendments may reduce this phenomenon (Franco-Andreu et al., 2016b) because of the higher water retention by the OM from organic amendments. In this study, the dominance of Gram-negative bacteria irrespective of irrigation could also be explained by the fact the drought conditions of non-irrigated soils were not extreme, as was the case in the above references. The cumulative rainfall during the assay was 185.8 mm and irrigation was 70 mm (Fig. S1 in Supplementary material), so the irrigated soils received a total of 255.8 mm of water, 38% more than the non-irrigated soils.

In the case of non-irrigated soils, the application of herbicides did not have any significant effects on the relative populations of Gram-negative and Gram-positive bacteria during the assay (Fig. 1). However, herbicides tended to increase the relative abundance of *Actinobacteria* after the second application (69–215 days) in S—H, S + GC1-H and S + GC2-H (Fig. 1). Baxter and Cummings (2008) have described the changes in soil microbial structure after three consecutive applications of the herbicide bromoxynil and, what's more, at high doses. The change in bacteria structure prompted a significant decrease in the Gram-negative/total Gram-positive (sum of Gram-positive group and *Actinobacteria*) bacteria ratio between treatments with and without herbicides in S + GC1 and S + GC2 at the end of the assay (Fig. S4 in Supplementary material). Similar results were found in soils fumigated with imazethapyr (Zhang et al., 2010).

Results indicated that the residual concentrations of herbicides were positively correlated with the relative percentage of Gram-positive bacteria and fungi, and negatively correlated with *Actinobacteria* (Table 4). Therefore, the dissipation of herbicides negatively affected Gram-positive bacteria and fungi, whereas it enhanced the relative population of *Actinobacteria*. So, fungi behaved in the opposite way to *Actinobacteria*. After the first application of herbicides (28 days), the relative population of fungi in S—H and S + GC1-H was significantly lower than in S and S + GC1, although S + GC2 and S + GC2-H did not record significant differences (Fig. 1). At the end of the assay (215 days), the negative effects of herbicides on fungi abundance were significant ($p < 0.05$) for all the treatments, and produced a generalized increase in the bacteria/fungi ratio (Fig. S4 in Supplementary material). This suggests that fungi were sensitive to the herbicides triasulfuron and prosulfocarb. The opposite effect between fungi and bacteria (Santás-Miguel et al., 2018) was because of the significant increase in *Actinobacteria*, the minimal effects of herbicides on Gram-negative and Gram-positive bacteria, and the significant decrease in fungi (Fig. 1).

The irrigation of soils enhanced the effects of herbicides in unamended and amended soils. The relative abundance of Gram-negative bacteria between the first and second herbicide applications (28 days) was lower in S + GC2-I-H than in S + GC2-I, but there were no significant differences between S—I and S-I-H or between S + GC1-I and S + GC1-I-H (Fig. 2). The same behaviour was found after the second herbicide application at 69 and 97 days. At the end of the assay (215 days), nonetheless, the decrease in Gram-negative abundance was significant ($p < 0.05$) for all the soil treatments with herbicides (S-I-H, S + GC1-I-H and S + GC2-I-H). The effect on Gram-positive bacteria was only significant ($p < 0.05$) at the end of the assay in S + GC2-I, where the presence of herbicides increased the relative population of Gram-positive bacteria.

The relative percentage of Gram-negative bacteria was negatively correlated with the prosulfocarb residue (Table 5). In contrast, the relative percentage of Gram-positive bacteria was positively correlated with the prosulfocarb residue. Therefore, the presence of prosulfocarb could induce the substitution of Gram-positive bacteria by Gram-negative bacteria. The same phenomenon could be found between *Actinobacteria* (negatively correlated with the herbicide residues) and fungi (positively correlated with the herbicide residues). The relative population of *Actinobacteria* was significantly higher with herbicides in all the soil treatments (S-I-H, S + GC1-I-H and S + GC2-I-H) as happened in non-irrigated soils. Despite the significant differences found during the assay for all the treatments, the ratio Gram-negative/total Gram-positive bacteria (Fig. S5 in Supplementary material) only recorded significant differences between S + GC2-I and S + GC2-I-H. In this soil, as in non-irrigated soils, the application of herbicides decreased this ratio accordingly with the increased persistence of herbicides (higher DT_{50}).

In contrast to *Actinobacteria*, the effects of herbicides on fungi followed the opposite trend by decreasing their relative abundance over time in all soil treatments. Moreover, the fungal decrease was clearly reflected in the bacteria/fungi ratio (Fig. S5 in Supplementary material). The increase in this ratio at the end of the assay again reflected the bacteria – fungi antagonism. The negative effects of some herbicides on fungi have been previously reported (Martin-Laurent et al., 2003; Wu et al., 2014). However, other studies have shown the negative effects that some herbicides, including triasulfuron and prosulfocarb, have on fungi at the beginning of the incubation period, although the fungal population subsequently recovered (Cycoń et al., 2013; García-Delgado et al., 2018; Wang et al., 2015; Zhang et al., 2010). In this study, the significant decline ($p < 0.05$) in the relative abundance of fungi could be related to the consecutive applications of high doses of a mixture of two herbicides. In addition, between 0 and 69 days the GC-amended soils (S + GC1 and S + GC2) tended to decrease the relative abundance of fungi with respect to S, as previously described by García-Delgado et al. (2018) and Pose-Juan et al. (2017).

The microbiological changes in soils after the second application of herbicides could be responsible for the change in their degradation rates, which tend to be faster after the second application when the relative population of *Actinobacteria* is enhanced. These organisms are known to be good degraders of complex substrates (Pose-Juan et al., 2017).

3.3. Relationship between soil microbial structure, herbicides, organic amendments and irrigation regimes

The global impact of herbicides on soil microbial community was assessed by PCA including the results in non-irrigated (Fig. 3A, B and C) and irrigated (Fig. 3D, E and F) soils. A PERMANOVA analysis (Table S1 in Supplementary material) testing for the significance of the effects on the relative abundances of PLFAs of herbicide application, sampling time, soil treatments and their respective interactions revealed the statistical significance of the three factors in non-irrigated and irrigated soils.

With respect to non-irrigated soils, unamended and amended soils recorded different PCA profiles. The soil treatment was not significant, but the application of herbicides was statistically significant in all three soil treatments (S, S + GC1 and S + GC2) (Table S1). The application of herbicides in soil without compost (S—H) clearly enhanced the abundance of *Actinobacteria* and reduced the relative abundance of fungi and Gram-negative bacteria. S + GC1-H and S + GC2-H were related to a low relative abundance of fungi and a high relative abundance of *Actinobacteria* and Gram-negative bacteria. In contrast, amended soils without herbicides were related to a high relative abundance of fungi and Gram-positive bacteria. In non-irrigated soils, therefore, herbicides had a clearly negative effect on fungi and Gram-positive bacteria, while promoting the relative abundance of *Actinobacteria*, irrespective of compost use. S—H was related to high relative abundance of Gram-negative bacteria, mainly at 124–215 days, while S + GC1-H and S + GC2-H recorded a closer relationship with Gram-negative bacteria than S + GC1 and S + GC2 (Fig. 3A, B and C). In a laboratory assay, soil fumigated with another sulfonylurea herbicide, azimsulfuron, enhanced microbial diversity by detecting different Gram-negative bacteria that were not found in a non-fumigated soil (Valle et al., 2006). S + GC1-H and S + GC2-H produced slower triasulfuron degradation than S—H, so the microbial shift may be deeper in the former.

The microbiology of non-irrigated soils was exposed to three clear stress factors, namely, high doses of triasulfuron and prosulfocarb, two

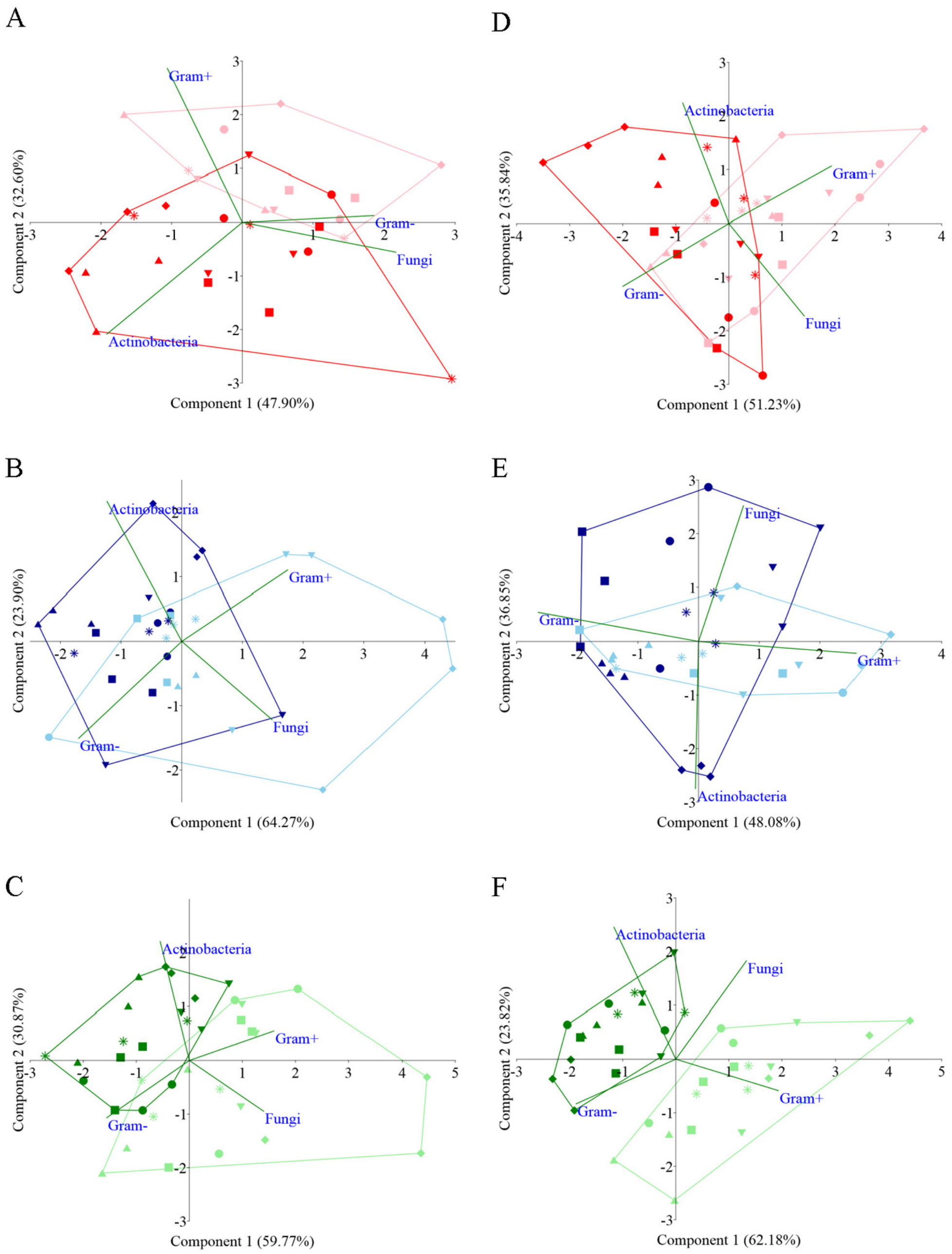

Fig. 3. Principal components analysis (PCA) of non-irrigated (A, B, C) and irrigated (D, E, F) soils showing loading scores for Gram− and Gram+ bacteria, *Actinobacteria* and fungi, and the scores of sampling times (0 days: circle; 28 days: inverse triangle; 69 days: square; 97 days: star; 124 days: triangle; 215 days: diamond) on the two main components. The application of herbicides is denoted by dark colors, the non-application of herbicides by light colors in unamended soil (Red), GC1-amended soil (Blue) and GC2-amended soil (Green). Percent variability explained by each principal component is shown in parentheses after each axis legend.

consecutive applications, and drought conditions. In this respect, the toxic effects of pesticides can be enhanced, with negative effects on microbial composition, enzyme activity and pesticide degradation (Franco-Andreu et al., 2016a). This could be the cause of the significant impact triasulfuron and prosulfocarb have on the microbial structure of unamended and GC-amended soils, as reported here. In contrast, a previous study under similar conditions but with lower herbicide doses revealed an impact on the microbial structure of unamended soil but not on the microbial structure of GC-amended soil (García-Delgado et al., 2018). Therefore, the capacity of organic amendments to buffer the effects of herbicides on the microbial structure could be limited by herbicide doses or consecutive applications.

The three soil treatments in irrigated soils had significant effects (Table S1 in Supplementary material) on the soil microbial structure. Unamended and GC1 amended soils (with and without herbicides) had a similar distribution in PCA with no significant relationship with any factor (Fig. 3D and E). In contrast, GC2 amended soils (with and without herbicides) had a strong relationship with *Actinobacteria* and a weak one with fungi (Fig. 3F). The negative effects of GC or other organic amendments on fungal abundance has previously been reported at both laboratory scale (Pose-Juan et al., 2017) and field scale (García-Delgado et al., 2018).

The application of herbicides in irrigated soils had some similarities with non-irrigated soils. The soil microbial structure of irrigated soils after herbicide application shifted towards a higher proportion of *Actinobacteria* and a lower relative amount of fungi, as was the case in non-irrigated soils. The presence or absence of herbicides and their interaction over time was significant in unamended soil. S—I was related to a high proportion of Gram-positive bacteria and fungi. In contrast, S-I-H evolved from points related to fungi towards a clear relationship with *Actinobacteria* and Gram-negative bacteria at the end of the assay. The herbicide factor was not significant in S + GC1 treatment. However, the time factor and the herbicide * sampling time interaction were significant. In fact, the evolution of S + GC1-I-H (dark blue symbols in Fig. 3) tends to be more closely related to *Actinobacteria* and less so to fungi over time. In contrast, the changes in microbial structure over time in S + GC1 seem to be related to the variation in the proportion of Gram-negative and Gram-positive bacteria because of the dispersion of points in component 1 and the low values of points in component 2, being closely related to fungi and *Actinobacteria*. The application of herbicides in GC2-amended soils and their interaction with sampling time were significant. PCA showed a clear differentiation between S + GC2-I and S + GC2-I-H. S + GC2-I was related to a high relative abundance of Gram-positive bacteria, mainly at 215 days. In contrast, S + GC2-I-H was related to a high relative abundance of *Actinobacteria* and Gram-negative bacteria. In addition, there was a clearly negative relationship with the abundance of fungi in both, S + GC2-I and S + GC2-I-H treatments, as described above.

After herbicide application, all the soils, irrespective of irrigation conditions, tended to enhance the relative population of *Actinobacteria*, mainly after the second application of herbicides, when their DT_{50} values were lower than the first one. The remaining triasulfuron concentration 69 days after the second application recorded lower concentrations than in the first application. Dissipation therefore increased in the second application, when *Actinobacteria* increased their relative abundance. In contrast, the remaining concentrations of prosulfocarb were higher after the second application. It therefore seems clear that a high relative abundance of *Actinobacteria* plays a key role in herbicide dissipation, being positive in the case of triasulfuron and negative in the case of prosulfocarb degradation.

4. Conclusions

The dissipation of triasulfuron and prosulfocarb in an agricultural soil under field conditions is influenced by the type and amount of green compost applied to the soil and by the irrigation regime. Two consecutive applications of triasulfuron increase the dissipation rate of this herbicide, although in the case of prosulfocarb it produces an accumulation of residual herbicide after the second application. A positive correlation between the amounts of herbicide residues and the total microbial population led to a decrease in the microbial population during the dissipation of herbicides and to a certain toxicity of herbicides for the microbial community. The microbial structure of unamended and GC-amended soils is modified after two consecutive applications of the herbicides triasulfuron and prosulfocarb. Herbicides increase the relative population of *Actinobacteria* and reduce the relative population of fungi compared to the initial situation in all the conditions studied. *Actinobacteria* seems to be responsible for the increase in the of degradation rate of triasulfuron after the second application.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.07.395.

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Material Suplementario

SUPPLEMENTARY MATERIAL

INFLUENCE OF DIFFERENT AGRICULTURAL MANAGEMENT PRACTICES ON SOIL MICROBIAL COMMUNITY OVER DISSIPATION TIME OF TWO HERBICIDES

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Number of Figures: 3

Soil sampling and sample processing

Surface soil samples from 0 to 10 cm were collected on the first day after herbicide application (0 days), and at different times up to 215 days after treatment to determine herbicide dissipation and at 0, 28, 69, 97, 124 and 215 days to determine soil phospholipid fatty acids (PLFAs) in all the treatments. Five sub-samples were taken in each plot, mixing them before they were transferred to polypropylene bottles. All the samples were transported to the laboratory in portable refrigerators. The soil water content of the bulk sample for each 10-cm soil layer was gravimetrically determined by weight difference, measuring the soil sample mass before and after drying at 110ºC for 24 h. Soil samples, previously air-dried overnight if necessary, were then sieved (< 2 mm) and their characteristics determined by standard methods (Sparks, 1996). The pH of the soils was determined in soil/water suspensions (1:2.5). The organic carbon (OC) content was determined by the modified Walkley-Black method. The dissolved organic carbon (DOC) content was determined in a suspension of soil in deionized water (1:2) after shaking (24 h), centrifugation (20 min at 10000 rpm), and filtering using a Shimadzu 5050 organic carbon analyser (Shimadzu, Columbia, MD, USA). Total N content was determined by the Kjeldahl method. Particle size distribution was determined using the pipette method. Soil samples to determine herbicide dissipation were analyzed immediately. To determine PLFAs, the samples were frozen at -80ºC and lyophilized prior to extraction and analysis.

Herbicide extraction and analysis

Duplicate subsamples of moist soil (6 g) from each plot were transferred to a glass tube, and extracted with methanol (12 mL). The samples were sonicated for 1 h, shaken at 20ºC for 24 h, and then centrifuged at 5045 g for 15 min. A volume of 8 mL was transferred to a clean glass tube and evaporated until dryness at 25ºC under a nitrogen stream using an EVA-EC2-L evaporator (VLM GmbH, Bielefeld, Germany). The residue was dissolved in 0.5 mL of methanol+formic acid (1%), filtered to remove particles > 0.45 µm in a GHP Acrodisc filter (Waters Corporation), and transferred to a HPLC glass vial for analysis (Marín-Benito et al., 2018).

The analysis of triasulfuron and prosulfocarb was performed by HPLC. The apparatus used was a Waters chromatograph (Waters Assoc., Milford, MA, USA), equipped with a model e2695 multisolvent delivery and autosampler system attached to a ZQ mass spectrometer detector (MS), with Empower software as the data acquisition and processing system. A Luna® 3 µm PFP(2) 100 Å (150 × 4.6 mm) column by Phenomenex (Torrance, CA, USA) was used at ambient temperature, and the mobile phase was acetonitrile:water+1% formic acid (70:30). The flow rate of the mobile phase was 0.4 mL min^{-1}, and the sample injection volume was 10 µL. Detection involved monitoring the positive molecular ion [m/z] 402.8 $[M+H]^+$ (triasulfuron) and 252.4 $[M+H]^+$ (prosulfocarb) after applying an optimized cone voltage of 20 V, and the retention times were 6.1 min and 14.1 min, respectively (Marín-Benito et al., 2018).

The matrix-matched calibration standards were between 0.1 and 2.5 µg mL^{-1} for both herbicides, and the limit of detection (LOD) and limit of quantification (LOQ) were in the ranges 0.018-0.026 µg mL^{-1} or 0.059-0.088 µg mL^{-1} for triasulfuron, and 0.005-0.008 µg mL^{-1} or 0.017-0.027 µg mL^{-1} for prosulfocarb in the unamended and amended soils, respectively. The method's recoveries were determined by spiking three unamended and amended soil samples with triasulfuron (76.9 µg kg^{-1}) or prosulfocarb (3.46 mg kg^{-1}) and performing the extraction procedure as described above. The mean recoveries of triasulfuron and prosulfocarb were >80% for the unamended and GC-amended soils. The amounts of triasulfuron and prosulfocarb extracted from soils were not corrected for recovery values because matrix-matched calibration method was used (Marín-Benito et al., 2018).

Dissipation kinetics

The dissipation kinetics for both herbicides was fitted to a single first-order (SFO) kinetic model ($C = C_0\ e^{-kt}$) or first order multicompartment (FOMC) model ($C = C_0/(t/\beta) + 1)^{\alpha}$), known also as the Gustafson and Holden model. FOCUS work group guidance recommendations were followed (FOCUS, 2006) for selecting the kinetic model that best describes the dissipation results. The coefficient of determination (R^2) and the chi-square (χ^2) test were calculated as indicators of the goodness of fit. The χ^2 test considers the deviations between observed and calculated values relative to the uncertainty of the measurements for a specific fit, and was used to compare the goodness of fit of the two models tested. The error value at which the χ^2 test is fulfilled at a given degree of freedom should be below 15% (at a 5% significance level). Values for the time to 50% dissipation (DT_{50}) were used to characterize the decay curves and compare variations in dissipation rates. The parameters of the kinetic models were estimated using the Excel Solver add-in package (FOCUS, 2006).

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Table S1. Results of PERMANOVA of the effect of herbicides application (H), sampling time (T), soil treatment (ST) and their interaction on the relative abundance (%mol) of PLFAs specific for Gram positive and negative bacteria, *Actinobacteria* and fungi in unamended and amended soils.

Non-Irrigation			**Irrigation**		
	F-value	*p*-value		F-value	*p*-value
Soil			**Soil**		
Herbicide	8.811	**0.0001**	Herbicide	7.235	**0.0017**
Time	0.9423	0.5235	Time	1.192	0.3059
H * T	-0.1369	0.4663	H * T	2.247	**0.0340**
GC1			**GC1**		
Herbicide	5.772	**0.0127**	Herbicide	3.071	0.0664
Time	3.710	**0.0057**	Time	4.741	**0.0004**
H * T	0.5160	**0.0306**	H * T	1.627	**0.0009**
GC2			**GC2**		
Herbicide	6.520	**0.0082**	Herbicide	32.87	**0.0001**
Time	3.103	**0.0092**	Time	0.6189	0.7198
H * T	1.977	0.0811	H * T	4.175	**0.0044**
Global			**Global**		
Herbicide	15.37	**0.0001**	Herbicide	26.69	**0.0001**
Time	5.067	**0.0001**	Time	2.819	**0.0061**
ST	3.409	0.0110	ST	6.398	**0.0003**
H * T	1.899	**0.0060**	H * T	3.124	**0.0001**
H * ST	-0.8854	0.2920	H * ST	3.123	**0.0021**
T * ST	0.2592	0.2448	T * ST	-0.7416	0.7282

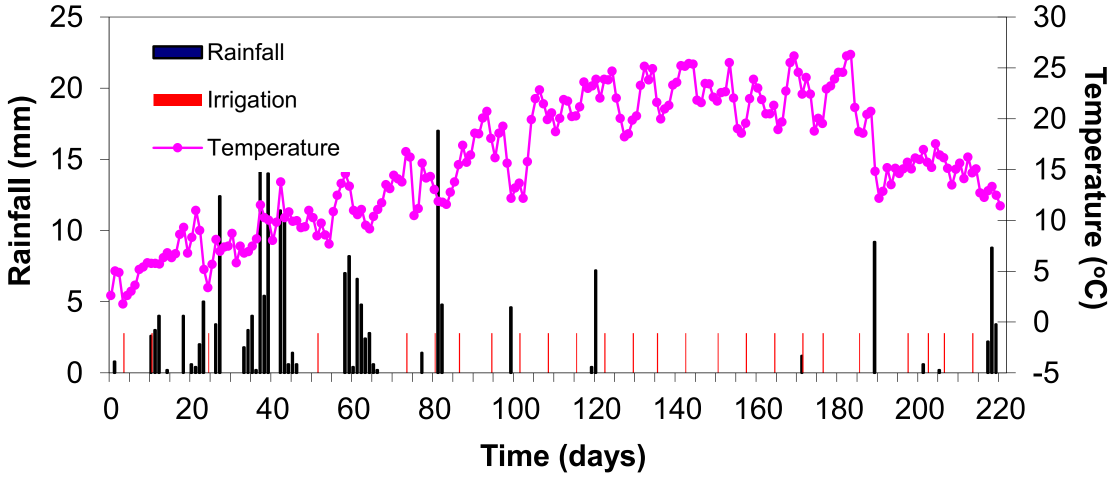

Fig. S1. Rainfall, additional irrigation and temperature evolution over time of experiment.

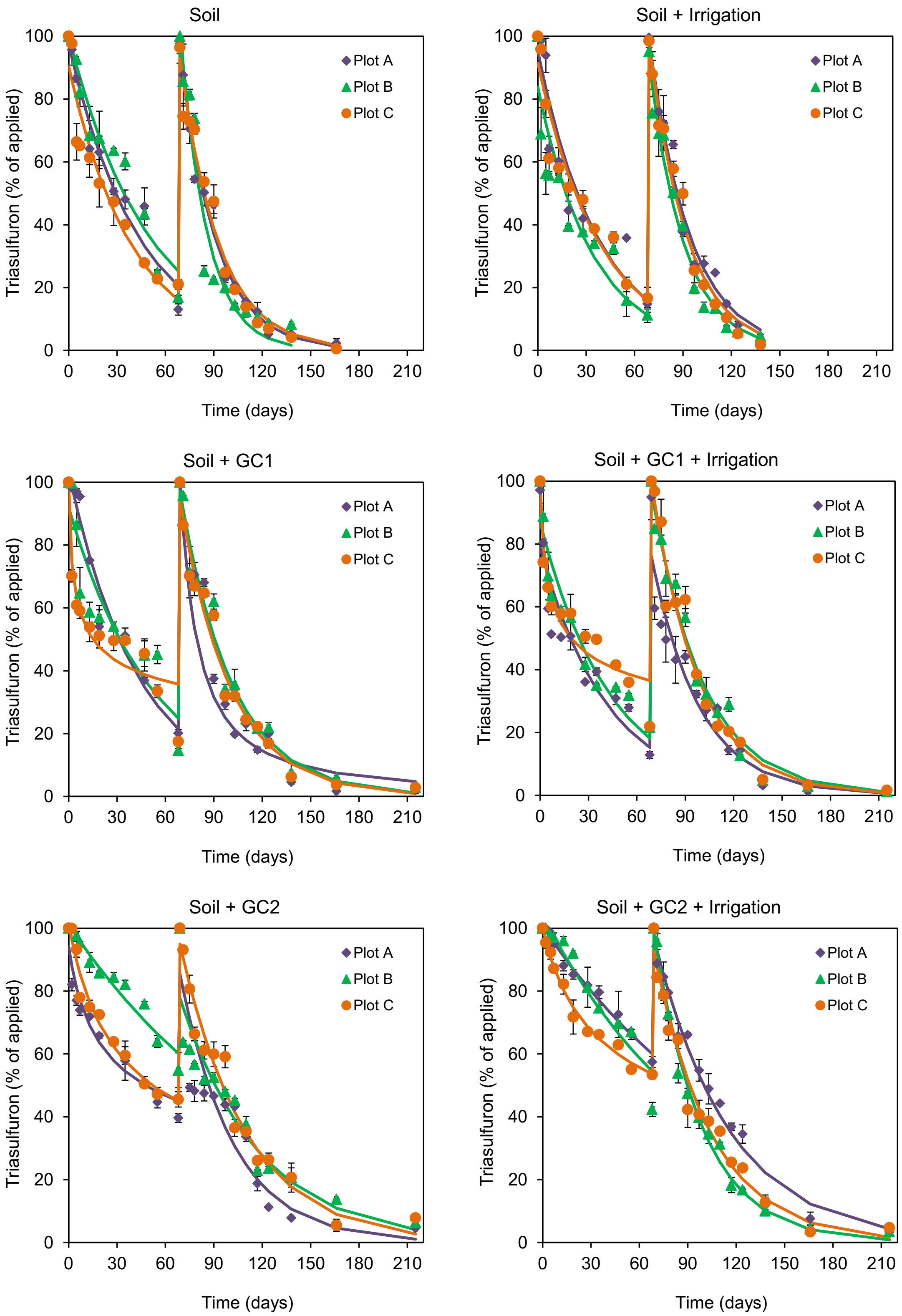

Fig. S2. Dissipation kinetics of triasulfuron in unamended soil and soil amended with green compost (GC1 and GC2) after the first application (0 - 68 days) and after the second application (69 - 215 days). Error bars represent the standard deviation of the mean values of plots treated with the herbicide (n=3).

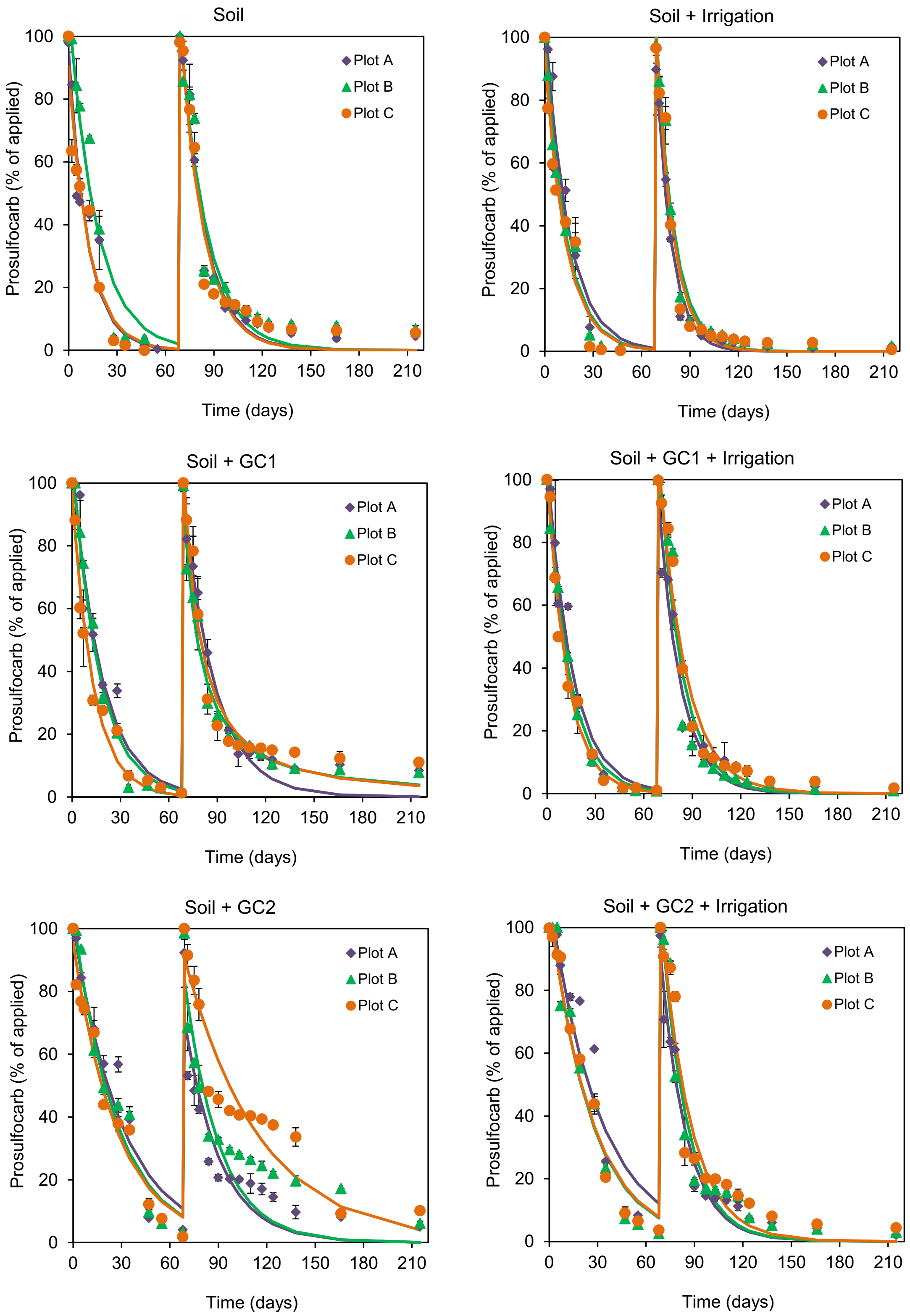

Fig S3. Dissipation kinetics of prosulfocarb in unamended soil and soil amended with green compost (GC1 and GC2) after the first application (0 - 68 days) and after the second application (69 - 215 days). Error bars represent the standard deviation of the mean values of plots treated with the herbicide (n=3).

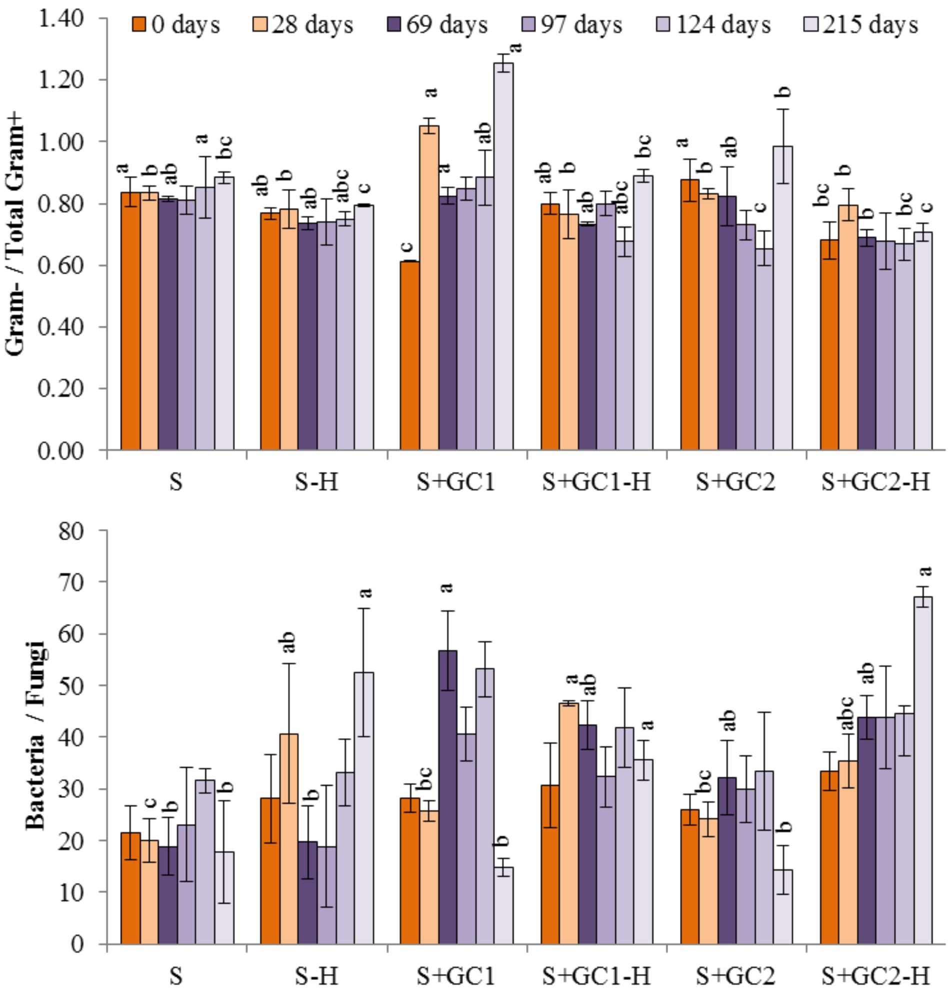

Fig S4. Ratio Gram- / total Gram+ bacteria (Gram+ bacteria and *Actinobacteria*) and bacteria / fungi in non-irrigated soils. Vertical bars represent the standard deviation of three replicates. Different letters indicate significant differences among treatments at the same sampling time (Tukey post hoc test, $p < 0.05$). S: unamended soil; S+GC1: amended soil with green compost 1; S+GC2: amended soil with green compost 2; H: herbicides application. The first application of herbicides is denoted by brown colour and the second application by purple colour.

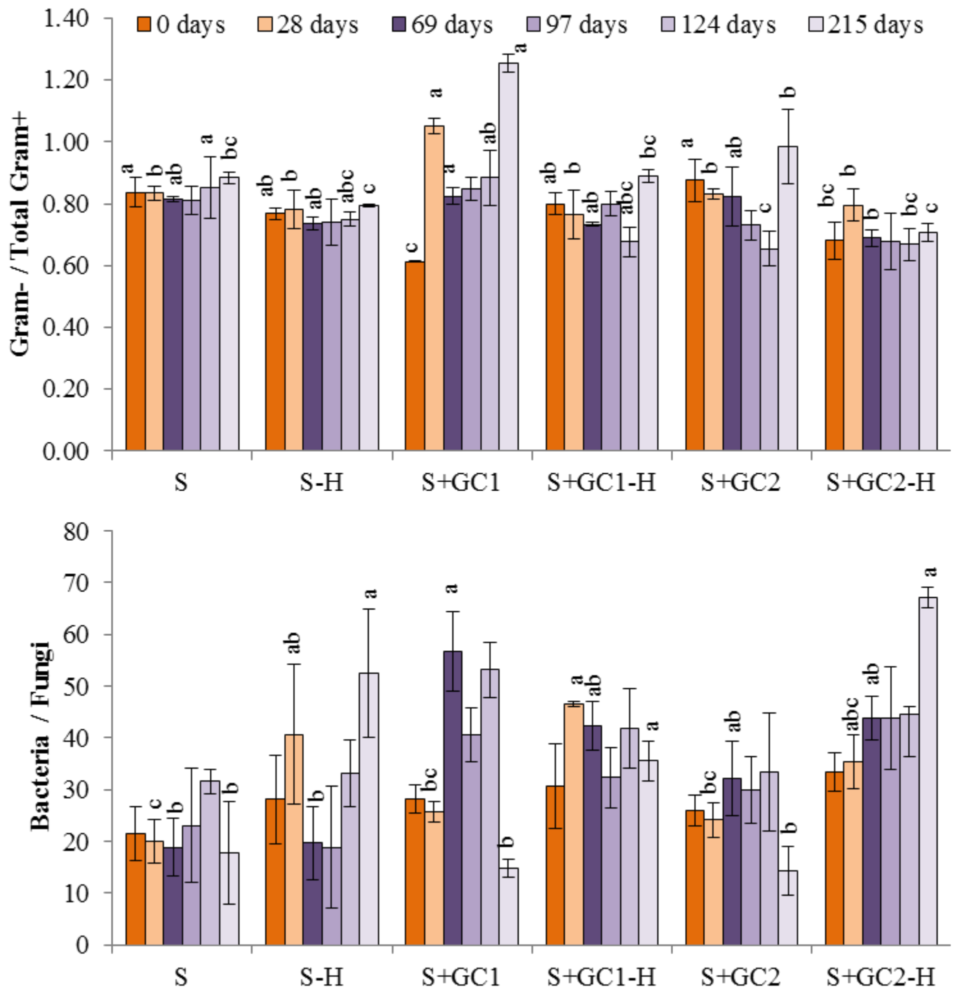

Fig S5. Ratio Gram- / total Gram+ bacteria (Gram+ bacteria and *Actinobacteria*) and bacteria / fungi in irrigated soils. Vertical bars represent the standard deviation of three replicates. Different letters indicate significant differences among treatments at the same sampling time (Tukey post hoc test, $p < 0.05$). S: unamended soil; S+GC1: amended soil with green compost 1; S+GC2: amended soil with green compost 2; H: herbicides application; I: irrigation. The first application of herbicides is denoted by brown colour and the second application by purple colour.

CAPÍTULO 5

Resumen

CAPÍTULO 5: Assessment of ^{14}C-prosulfocarb dissipation mechanism in soil after amendment and its impact on the microbial community

RESUMEN

El objetivo de este trabajo fue estudiar los mecanismos de disipación y el balance de masa de prosulfocarb en un suelo sin enmendar y enmendado con compost vegetal (GC) en condiciones de laboratorio con el fin de ampliar la información obtenida sobre la disipación de prosulfocarb en los ensayos de campo previamente estudiados. El uso de enmiendas orgánicas en suelos para mejorar su contenido en carbono orgánico (CO) es una práctica usada de manera global en la agricultura moderna. Sin embargo, este aporte de CO al suelo introducido por la aplicación de las enmiendas puede modificar el comportamiento fisicoquímico y la biodisponibilidad de los herbicidas en esos suelos. Estos cambios en su biodisponibilidad junto a la presencia de nuevos microorganismos procedentes de las enmiendas pueden afectar a la disipación del herbicida.

En este trabajo, el herbicida prosulfocarb sin marcar y marcado en ^{14}C (~ 100 Bq g^{-1}) se aplicó en dos concentraciones (4 y 10 mg kg^{-1}) en un suelo sin enmendar y enmendado con GC a una dosis de 180 t ha^{-1} (20% en peso seco). Se determinó la disipación y el balance de masa de ^{14}C-prosulfocarb aplicado a partir de las extracciones secuenciales con solución acuosa (0.01M $CaCl_2$) y solución orgánica (metanol) (fracciones extraíbles) y la determinación de las fracciones mineralizada y remanente o no extraíble de ^{14}C-prosulfocarb por combustión. Además, se evaluó la actividad deshidrogenasa (DHA) y el perfil de ácidos grasos de fosfolípidos (PLFA) del suelo para conocer el efecto de los residuos del herbicida sobre la actividad y estructura de las comunidades microbianas durante el periodo de disipación del mismo.

La cinética de degradación del prosulfocarb no marcado registró una fase *lag* inicial de 9 días en el suelo enmendado y sin enmendar cuando se aplicó a la dosis alta, y una fase *lag* de 14 días en el suelo enmendado cuando se aplicó a la dosis baja. A esta fase *lag* le siguió una degradación más rápida que se ajustó a un modelo cinético de primer orden (SFO). La velocidad de disipación de prosulfocarb disminuyó en el suelo enmendado debido a una mayor adsorción del herbicida. Los valores de vida media (DT_{50}) fueron más elevados en el suelo enmendado (16.2-21.6 días) que en el suelo sin enmendar (8.0-13.9 días) y estos valores aumentaron al aumentar la concentración de prosulfocarb en el suelo sin enmendar, pero disminuyeron en los suelos enmendados.

También se ha observado un aumento o disminución de los valores de DT_{50} para otros compuestos con un aumento de la concentración aplicada, siendo este comportamiento explicado por las diferentes fases *lag* implicadas en el proceso de disipación. La disipación de prosulfocarb fue mucho más lenta en el suelo esterilizado indicando que la degradación microbiana es relevante en este proceso.

El balance de masa total fue > 80% cuando la disipación del herbicida marcado fue próxima al 50% en el suelo enmendado y sin enmendar, y disminuyó hasta un 60%-70% al final del experimento. Esta disminución puede atribuirse a procesos paralelos que contribuyen a la disipación de compuesto como la volatilización. Estos resultados se corresponden con los elevados porcentajes resultantes de la suma de las fracciones extraíbles en agua y en metanol obtenidos en el periodo transcurrido hasta alcanzar el 50% de disipación del herbicida. A partir de este periodo la fracción mineralizada del ^{14}C-prosulfocarb aumentó en el rango 11%-31% así como la fracción residual o no extraíble del compuesto (hasta un 35%-44%) en suelos sin enmendar y enmendados con GC. Los resultados pusieron de manifiesto una mayor biodisponibilidad de prosulfocarb en el suelo sin enmendar que en el enmendado de acuerdo con la fracción extraíble más elevada en agua. Por otra parte, la disminución en las cantidades extraíbles de herbicida desde el suelo sin enmendar y enmendado con el tiempo fue consecuencia del envejecimiento del herbicida en el suelo, aumentando la fracción residual del compuesto fuertemente enlazado en el suelo. Estos residuos podrían ser inmovilizados mediante interacciones fisicoquímicas entre el compuesto y los componentes del suelo, especialmente el CO al aumentar el periodo de incubación.

La DHA del suelo disminuyó en los suelos sin enmendar y se mantuvo en los suelos enmendados hasta el final del ensayo. La estructura microbiana apenas fue alterada durante el proceso de degradación del prosulfocarb. Al final del ensayo, el herbicida no tuvo ningún efecto significativo en la abundancia relativa de bacterias Gram negativas y Gram positivas y hongos independientemente de la enmienda o la dosis de herbicida. Sólo en el caso del suelo sin enmendar tratado con la dosis más alta de herbicida (10 mg kg^{-1}) se observó un descenso de la población relativa de *Actinobacterias*. En el suelo enmendado este efecto fue amortiguado por el GC.

Artículo

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Assessment of ^{14}C-prosulfocarb dissipation mechanism in soil after amendment and its impact on the microbial community

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ABSTRACT

Adding organic amendments to soil could modify the bioavailability of herbicides and lead to changes in the microbial community's activity and structure. The objective here was to study the dissipation and total mass balance of ^{14}C-labeled prosulfocarb applied at two rates (4 and 10 mg kg^{-1}) in unamended and green compost (GC)-amended soil. Soil dehydrogenase activity (DHA) and phospholipid fatty acid (PLFA) profile analysis were determined to evaluate the effect of herbicide residues on microbial community's activity and structure over the dissipation period. The dissipation rate of prosulfocarb decreased after soil amendment due to higher herbicide adsorption by the amended soil. The 50% dissipation time (DT_{50}) increased 1.7 times in the unamended soil when the concentration of prosulfocarb increased 2.5 times. The mass balance results indicate that the sum of water and organic extractable fractions represented the highest amounts up to the dissipation of 50% ^{14}C-prosulfocarb. The ^{14}C-herbicide was then mainly mineralized (up to 11%-31%) or formed non-extractable residues (up to 35%-44%). The amount of ^{14}C-prosulfocarb residues extracted with methanol was slightly higher in amended soils than in unamended ones. ^{14}C-prosulfocarb mineralization was higher in unamended soils than in amended ones. The formation of non-extractable residues was continuous, and increased over time. Soil DHA decreased in the unamended soil and was maintained in the GC-amended soil at the end of the assay. The microbial structure was barely disturbed over the prosulfocarb degradation process, although it was clearly influenced by the application of GC. The results obtained reveal the influence organic amendment has on herbicide bioavailability to decrease its biodegradation and buffer its impact on the soil microbial structure.

1. Introduction

Prosulfocarb (S-benzyl dipropyl thiocarbamate) is a selective pre- and early post-emergence herbicide used for weed control in different crops (EFSA, 2007; Scherner et al., 2018). It is absorbed by leaves and roots, and its modus operandi includes inhibiting the synthesis of long-chain fatty acids (EFSA, 2007). Prosulfocarb records low solubility in water and high hydrophobicity with high adsorption (Kd = 11.7-32.8 mL g^{-1}), low mobility and moderate persistence in soils (EFSA, 2007; PPDB, 2018). Half-life (DT_{50}) values for this herbicide ranged from 6.3 to 38.4 days for different soils and different conditions (Braun et al., 2017; Gennari et al., 2002; PPDB, 2018; Scherner et al., 2018). The dissipation of this herbicide is due mainly to microbial mineralization, the formation of non-extractable residues, the formation of the minor soil metabolite prosulfocarb sulfoxide and volatilization (Braun et al., 2017; EFSA, 2007; Gennari et al., 2002).

A significant correlation has been reported between the adsorption constants of prosulfocarb by soils and soil organic carbon (OC) contents (Nègre et al., 2006). This effect has also been observed in a field experiment carried out in soil amended with the organic residue green compost (GC), where prosulfocarb adsorption increased in the topsoil and a significant correlation coefficient was found between residual herbicide concentrations and OC content in the GC-amended soil profile (García-Delgado et al., 2019; Marín-Benito et al., 2018a,b). In this experiment, the dissipation of herbicide was relatively rapid, with DT_{50} ranging between 7.1 and 20.3 days under different conditions (soil amendment rate, irrigation regime and herbicide rate) (García-Delgado et al., 2019). However, significant residual amounts of prosulfocarb were detected in the topsoil and through the soil profile after five months of soil treatment with the herbicide, especially in the GC-amended soil (Marín-Benito et al., 2018b). Prosulfocarb residues could remain in the soil if herbicide adsorption increases over time, which

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would lead to an herbicide aging process in the soil, as indicated for other organic contaminants (Barriuso et al., 2008; Kästner et al., 2014) and lower bioavailability for degradation by microorganisms. The aging process depends on soil and compound properties, and soil organic matter (OM) is reported to be an important factor involved in its development (Gevao et al., 2003).

The application of organic amendments to the soil is a common agricultural practice for increasing nutrients and OC content. They could also act as a barrier to avoid the spread or leaching of pesticides, potentially providing environmental protection (Ferreira Mendes et al., 2019). This practice boosts soil properties and improves soil microbial activity (García-Delgado et al., 2018; Zornoza et al., 2016). However, the OM provided by the amendments could also lead to changes in the physicochemical behaviour of the pesticides applied in amended soils and affect their degradation, persistence or mobility (Marín-Benito et al., 2017).

Changes in these processes could occur if the bioavailability of compounds is modified in the soils amended with organic residues. Marín-Benito et al. (2012, 2014) have reported the effect that organic residues applied as soil amendments have on the bioavailability of different pesticides. Different dissipation mechanisms have been described for compounds such as linuron, diazinon or myclobutanil, with different characteristics in soils amended with sewage sludge, grape marc and spent mushroom substrate (SMS). The effect of soil aging gives rise to lower extractable amounts for diazinon and an increase in non-extractable residues for linuron. Similar effects have also been recorded for tebuconazole and cymoxanil when applied to SMS-amended soil at two rates (Álvarez-Martín et al., 2016) or for isoproturon in soils amended with biochar (Reid et al., 2013). Studies focusing on the influence GC has on the bioavailability of herbicides are scarce despite the influence this residue has on increasing the sorption or decreasing the dissipation of different herbicides in GC-amended soils (Pose-Juan et al., 2018).

The addition of organic amendments to soil could lead to changes in the microbial community's activity and structure. A stimulatory effect on soil microbial activity determined by the increase in dehydrogenase activity (DHA) has usually been observed in soils after the application of different organic residues (Álvarez-Martín et al., 2016; Marín-Benito et al., 2014; Pose-Juan et al., 2018). The amendment's positive effect on soil microbial activity is due to the greater OC content available in the amended soil. At the same time, the presence of new microorganisms from these residues could affect the soil's natural microbial communities. However, these effects could be different in the presence of herbicides in the amended soils. It has been reported that soil DHA could decrease or increase over time in the presence of herbicides depending on their dissipation and/or metabolism in the amended soil (Pose-Juan et al., 2017). Furthermore, the combined application of organic amendments and herbicides could increase or decrease the relative abundance of bacteria and fungi over herbicide dissipation time in amended soils depending on the type of amendment and the time of incubation (García-Delgado et al., 2018, 2019; Pose-Juan et al., 2017). These effects may be influenced by the bioavailability of herbicide in the amended soils, although it has hardly been studied.

Taking into account the rapid dissipation of prosulfocarb in surface soil revealed in previous field studies (García-Delgado et al., 2019; Marín-Benito et al., 2018a), which was mainly due to processes of sorption, degradation and leaching, it has been considered of interest to assess the dissipation mechanisms of prosulfocarb when applied to unamended and amended soils. Braun et al. (2017) have reported results on the fate of prosulfocarb in a soil and a sediment-water system, but this study was conducted over too short a time period to obtain conclusions on the dissipation mechanism, and the soil organic amendment's influence was not studied.

The objective here was to study the dissipation and total mass balance of ^{14}C-labeled prosulfocarb in an unamended soil and after the application of GC as organic amendment. Accordingly, a laboratory experiment was set up to determine the mineralized, extractable (as parent or metabolites) and non-extractable (bound residues) fractions of ^{14}C-prosulfocarb over time. At the same time, soil DHA and phospholipid fatty acid (PLFA) profile analysis were performed to evaluate the effect of herbicide residues on soil microbial activity and structure over the dissipation period in unamended and GC-amended soils. The herbicide rate applied to soils was one of the variables considered. In view of the high use of prosulfocarb in agriculture, the outcomes obtained could help to explain the bioavailability of aged residues of prosulfocarb in soils and their potential environmental impact after new soil management.

2. Materials and methods

2.1. Chemicals

The non-labeled prosulfocarb (PESTANAL®, > 98.9% purity) was supplied by Sigma-Aldrich Química S.A. (Madrid, Spain). The labeled [ring-U-^{14}C] prosulfocarb (specific activity 3.16 MBq mg^{-1} and 94.8% purity) was supplied by IZOTOP Co. Ltd (Budapest, Hungary). Prosulfocarb has a water solubility of 13.2 mg L^{-1} (20°C) and a log K_{ow} of 4.48 (pH 7, 20°C) (PPDB, 2018).

HPLC grade acetonitrile, methanol and anhydrous chloroform (> 99% purity) were supplied by VWR International Eurolab (Spain), and 2,3,5-Triphenyltetrazolium chloride (TTC) and 2,3,5-triphenylformazan (TPF) were supplied by Sigma-Aldrich Química S.L. (Madrid, Spain).

2.2. Organic amendment

The organic residue green compost (GC) supplied by the nursery EL Arca (Salamanca, Spain) was used after composting. It was generated from the pruning of plants in gardens and parks. The characteristics of the GC (on a dry weight basis) are as follows: pH 7.2, total OC content 24.1%, dissolved organic carbon (DOC) content 0.703%; total N content 1.1%, C/N ratio 21.8, ash percentage 54%, and moisture content was 48.6%. These characteristics were determined in samples previously homogenized and sieved (< 2 mm). The pH was determined in a residue/water suspension (1:2.5). Total OC and N contents were determined by a LECO CN628 elemental analyzer (LECO Corporation, Saint Joseph, MI, USA). DOC was determined in a suspension of residue in Milli-Q ultrapure water (1:2) after residue shaking (24 h at 20°C), centrifugation (20 min at 10000 rpm), and filtering (Minisart NY 25 filter 0.45 µm, Sartorius Stedim Biotech, Germany) using a Shimadzu TOC-VCSH total organic carbon analyzer (Shimadzu, Columbia, MD, USA). The ash percentage was determined by weight difference after ignition at 540°C for 24 h.

2.3. Unamended soil sampling and preparation of amended soil

The unamended soil (S) used in these experiments was a Typic Haploxerept with sandy clay loam texture (57.63% sand, 16.97% silt, 24.98% clay and 0.21% carbonate content; Table 1). There had been no application of prosulfocarb over at least the previous 5 years. It was

Table 1
Characteristics of unamended soil, green compost and green compost-amended soil.

Samples	pH	OC[a] (%)	DOC[b] (%)	N (%)	C/N
Soil	7.35	1.30	0.006	0.12	10.8
Green compost	7.20	24.1	0.703	1.11	21.8
Soil + Green compost	7.30	4.66	0.027	0.42	11.1

[a] Organic carbon.

[b] Dissolved organic carbon.

taken from the surface horizon (0–30 cm) at the Muñovela experimental farm (40°55'56" N latitude and 5°52'53" W longitude) belonging to the Institute of Natural Resources and Agrobiology of Salamanca (IRNASA-CSIC). The amended soil was prepared by uniformly mixing soil with GC (S + GC) at a rate of 20% w/w (180 t ha^{-1}) on a dry weight basis. The soil and GC were mixed after sieving (< 2 mm). The amended soils were then incubated for ten days under laboratory conditions (~20°C) to stabilize the organic residue. Unamended and amended soil subsamples were used in the dissipation experiment.

The unamended and amended soil characteristics of pH, total OC and N contents and DOC were determined in samples sieved (< 2 mm) and dried as previously indicated (Table 1). These soil's characteristics have been determined in Marín-Benito et al. (2018a).

2.4. Dissipation and analysis of unlabeled prosulfocarb

Herbicide dissipation experiments were conducted in duplicate samples of S and S + GC. A suitable concentration of unlabeled prosulfocarb was added to 700 g of S and S + GC to achieve herbicide concentrations of 4 mg kg^{-1} (agronomic dose) and 10 mg kg^{-1} (2.5 times the agronomic dose). The soils were incubated at 20°C in the dark and their moisture content was maintained at 40% of the maximum water-holding capacity throughout the study by adding sterile Milli-Q ultrapure water when needed. In addition, sterilized soil samples were prepared by autoclaving 300 g of S and S + GC at 120°C for 1 h over three consecutive days. The herbicide was applied to the sterilized samples to give a concentration of 4 mg kg^{-1}. These sterilized soil samples were used as controls to verify the herbicide's chemical degradation. Finally, S and S + GC soils were prepared for microbiological control by adding only sterile Milli-Q ultrapure water. All these soils were stirred with a sterilized spatula, and all the steps were performed in a sterile cabinet. Soil samples were taken at day 0 (24 h after prosulfocarb application) for herbicide analysis, and thereafter repeatedly at different time intervals up to 50 days, depending on the dissipation rate of prosulfocarb in each soil treatment.

Soil samples (2 x 6 g) of each duplicate treatment were taken at different times, and prosulfocarb was extracted. The samples were sonicated in glass tubes with 12 mL of methanol for 1 h and then shaken in a rotary shaker at 20°C for 24 h. The samples were centrifuged at 3000 rpm for 7 min, and the extracts were sieved in Minisart NY 25 filters (Sartorius Stedim Biotech, Germany) to remove particles > 0.45 µm. A volume of the liquid extract (8 mL) was evaporated until dryness at 25°C in an EVA-EC2-L evaporator (VLM GmbH, Bielefeld, Germany). The residue was dissolved in 0.75 mL of methanol with 1% formic acid and transferred to glass vials for analysis.

Prosulfocarb was determined by HPLC, equipped with a model e2695 multisolvent delivery and autosampler system attached to a ZQ mass spectrometer detector (MS) (Waters Assoc. Milford, MA, USA), as indicated by Marín-Benito et al. (2018a). The positive molecular ion (m/z) 252.4 $[MH]^+$ for prosulfocarb was monitored at 14.6 min, while the positive molecular ion (m/z) 267.4 $[MH]^+$ (prosulfocarb sulfoxide) was qualitatively monitored at 6.67 min (EFSA, 2007). The method's recoveries and detection and quantification limits have been determined in a previous work (Marín-Benito et al., 2018a).

2.5. Dissipation and analysis of ^{14}C labeled prosulfocarb

Duplicate samples of S and S + GC with ^{14}C labeled prosulfocarb were incubated at the same time as unlabeled prosulfocarb to study the herbicide's dissipation mechanism and its bioavailability. A volume of 10 mL of an aqueous solution of unlabeled herbicide was labeled with ^{14}C-prosulfocarb and added to 500g of S and S + GC to achieve concentrations of 4 mg kg^{-1} and 10 mg kg^{-1} of dry soil and an activity of approximately 100 Bq g^{-1}. The initial moisture content of both S and S + GC was also adjusted to 40% of their maximum water-holding capacity, as in the dissipation experiments with unlabeled herbicide. In these samples, a $^{14}CO_2$ trap (a vial containing 1 ml of NaOH 1M) was attached to the lid with a stainless-steel clip (Reid et al., 2002).

Labeled prosulfocarb was extracted from soil samples in a sequential process: first, 2 x 5 g of each duplicate treatment were extracted with 10 mL of a 0.01 M $CaCl_2$ Milli-Q ultrapure water solution by shaking for 24 h, and then a second extraction was performed with 10 mL of methanol by shaking for 24 h. The quantitative determination of ^{14}C-prosulfocarb was performed by liquid scintillation using a Beckman LS 6500 liquid scintillation counter (Beckman Instruments Inc., Fullerton, CA). The ^{14}C-activity of the solutions, associated to parent compound prosulfocarb and possible transformation compounds, was measured in disintegration per minute (dpm), being determined in duplicate in 1 mL of extract to which 4 mL of scintillation cocktail was added (Ecoscint TMA, National Diagnostics, Atlanta, GA). The remaining ^{14}C-activity in the soil was determined by combusting 1 g dried samples using a Biological Oxidizer (RJ. Harvey OX-500 Instrument Corporation, NJ) under excess O_2 at 900 °C. The $^{14}CO_2$ generated was trapped in a mixture of ethanolamine (1 mL) and scintillation cocktail (Oxysolve C-400, Zinsser Analytic, Berkshire, UK, 15 mL), and determined as indicated before. $^{14}CO_2$ from the mineralization of labeled prosulfocarb retained in 1 M NaOH (1 mL) in the scintillation vial was determined at different sampling times by mixing 4 mL of scintillation cocktail as previously indicated. The mass balance in ^{14}C residues was calculated as a percentage of the total amount of ^{14}C radioactivity obtained from the different fractions.

2.6. Soil dehydrogenase activity

Soil DHA was determined immediately after spiking prosulfocarb in the unamended and GC-amended soils (0 days) and 27 and 42 days after the application of the herbicide to measure overall microbial activity during the dissipation. Soil DHA was determined following the Tabatabai method (Tabatabai, 1994).

2.7. Phospholipid fatty acid (PLFA) profile analysis

PLFA was analysed as described in Pose-Juan et al. (2017) to evaluate the microbial community's composition and its evolution after different times during the dissipation of prosulfocarb in the soil. Samples were taken immediately after spiking prosulfocarb in the soil (0 days) and 27 and 42 days after the herbicide's application, and then freeze-dried, and 2 g of this material was used for lipid extraction. Lipids were extracted with a extractant consisted of 50 mM K_2HPO_4 in H_2O, methanol, and chloroform (1:2.5:1.25, v/v/v). Phospholipids were separated from non-polar lipids and converted to fatty acid methyl esters before analysis. Quantification was performed using an Agilent 7890 gas chromatograph (Agilent Technologies, Wilmington, DE, USA) equipped with a 25-m Ultra 2 (5% phenyl)-methylpolysiloxane column (J&W Scientific, Folsom, CA, USA) and a flame ionization detector (FID). PLFAs were identified using bacterial fatty acid standards and software from the Microbial Identification System (Microbial ID, Inc., Newark, DE, USA). Specific PLFAs (Zelles, 1999) were used as biomarkers to quantify the relative abundances of Gram negative (monounsaturated fatty acids and cyclopropyl 17:0 and 19:0) and Gram positive (iso and anteiso saturated branched fatty acids) bacteria, *Actinobacteria* (10-methyl fatty acids) and fungi (18:2 ω6 cis).

2.8. Data analysis

The dissipation curves for the herbicide were fitted to a single first-order (SFO) kinetic model and a first-order multi-compartment (FOMC) model. FOCUS work group guidance recommendations were followed (FOCUS, 2006) for selecting the kinetic model that best describes the dissipation results. The coefficient of determination (r^2) and the chi-square (χ^2) test were calculated as indicators of the goodness of fit. The error value at which the χ^2 test is fulfilled at a given degree of freedom should be below 15% (at 5% significance level). Values for the time to 50% dissipation, or DT_{50} values, were used to characterize the decay curves and compare variations in dissipation rates. The kinetic models' parameters were estimated using the Excel Solver add-in package (FOCUS, 2006).

Analysis of variance (ANOVA) was used to evaluate the effects of the different treatments on pesticide dissipation. Standard deviation (SD) was used to indicate variability among replicates, and the least significant difference (LSD), at a confidence level of 95%, was determined to evaluate the effects of different soil treatments on DT_{50} values. The Games–Howell post-hoc test at $p < 0.05$ was used to determine significant differences between means and evaluate the effects of the different soil treatments both at the same sampling time and at the sampling times within the same soil treatment on DHA and PLFAs. Pearson correlation coefficients between the remaining percentage of prosulfocarb, soil moisture content, and microbial structure and activity were determined to assess how these variables are related to each other. ANOVA and correlation analyses were conducted using the IBM SPSS Statistics v24 software package. Principal component analysis (PCA) was performed with PAST v3.15 software to determine the most meaningful variables and the global impact of the herbicide and GC on soil microbial communities.

3. Results and discussion

3.1. Dissipation of non-labeled prosulfocarb in unamended and amended soils

Fig. 1 shows the dissipation curves of non-labeled prosulfocarb applied at two doses (expressed as the percentage of the herbicide initially applied versus the incubation time) in S and S + GC. The study was carried out up to 50 days, when the amounts of herbicide remaining ranged from 2.72% to 3.10% in S, and from 10.1% to 5.66% in S + GC. The dissipation kinetics of prosulfocarb was fitted to SFO and FOMC models, and kinetic parameters were calculated for each treatment (Table 2).

The degradation kinetics of prosulfocarb applied at a high dose (10 mg kg^{-1}) recorded a lag phase of nine days (very slow degradation rate) in S and S + GC, and when applied at a low dose it recorded a lag phase of 14 days in S + GC. The existence of a lag phase shows the adaptation time that the microorganisms need to degrade the herbicide; it has been reported for the dissipation of several pesticides under laboratory conditions (Marín-Benito et al., 2012, 2014; Yuan et al., 2015). In general, this lag phase has been detected for hydrophobic pesticides, such as linuron, diazinon, pyrimethanil, and in amended soils, indicating it could be due to the initial sorption of pesticides by the amended soils (Guo et al., 2000). The lag phase was shorter in GC-amended soil for prosulfocarb applied at a high dose possibly due to a higher fraction of non-sorbed herbicide available for degradation than when applied at a low dose. In our case, this phase was followed by a faster degradation phase that fitted the SFO model better than the FOMC model (Table 2). The fitting of prosulfocarb dissipation to the SFO model has been reported in unamended soil, and to the FOMC model in amended soils (EFSA, 2007; Gennari et al., 1998; Marín-Benito et al., 2018a; Scherner et al., 2018). In a field study, the dissipation of prosulfocarb applied as a commercial formulation (Auros®) in unamended and GC-amended soils followed biphasic kinetics with a rapid first phase (as in the laboratory experiment) and then a slower and prolonged phase up to 100 days (García-Delgado et al., 2019; Marín-Benito et al., 2018a). The different kinetics models used to fit the dissipation curves in laboratory and field studies could be also due to the application of the herbicide as active ingredient or commercial formulation, respectively.

The prosulfocarb dissipation rate decreased after soil amendment. The DT_{50} values obtained were higher in S + GC (21.6-16.2 days) than in S (8.0-13.9 days) (Table 2). Nevertheless, these values are within the range reported for different soil types and different applications of this herbicide in laboratory or field studies (6.3-40.3 days) (EFSA, 2007; Gennari et al., 2002; Marín-Benito et al., 2018a). Prosulfocarb dissipation is always faster in S than in S + GC, with this being attributed to a higher prosulfocarb adsorption by the S + GC due to its higher OC content. Soil OC plays an important part in increasing the soil adsorption of hydrophobic pesticides like prosulfocarb. Significant correlation coefficients between adsorption parameters and soil OC content have been reported for prosulfocarb in unamended or amended soils with different OC contents (Marín-Benito et al., 2018b). The influence of adsorption on decreasing the dissipation rate of pesticides in soils is due to a decrease in the bioavailability and biodegradation of organic compounds adsorbed by the soil, and it has been observed for other pesticides such as diazinon and myclobutanil in soils amended with different organic residues, or for fungicides such as penconazole, metalaxyl and iprovalicarb in unamended and SMS-amended soils (Botterweck et al., 2014; Jaquet et al., 2014; Marín-Benito et al., 2012, 2014).

The herbicide dose's influence on its dissipation indicates that the DT_{50} value increased 1.7 times in S when the concentration of prosulfocarb increased 2.5 times. No general behaviour has been reported for other compounds. Increases in DT_{50} with the concentration of herbicide were observed for pethoxamid (Rodríguez-Cruz et al., 2019), but not for triasulfuron (Pose-Juan et al., 2017) when the herbicide concentration increased 5 and 25 times in unamended soil. In contrast to unamended soils, degradation was faster in S + GC when the highest concentration of prosulfocarb (10 mg kg^{-1}) was applied. A possible explanation for this behaviour could be the different lag phases involved in the dissipation process. Nevertheless, the DT_{50} values of prosulfocarb were higher in S + GC than in S when the highest dose was applied.

The dissipation of prosulfocarb applied at low dose in sterilized soils was also recorded in S and S + GC to evaluate the role the microbial community played in this process (Fig. 1). Dissipation was much slower in both sterilized unamended and amended soils than in non-sterilized ones. Prosulfocarb residues were 78.5% after 42 days of incubation in S and 70.3% in S + GC. These results indicate that microbial degradation plays an important role in prosulfocarb dissipation. The low degradation of prosulfocarb observed in sterilized soils was not attributed to photodegradation, as these soils were kept in the dark during the assay, and suggests the existence of other dissipation processes, such as the formation of non-extractable residues and volatilization over time (EFSA, 2007).

To check for the possible formation of degradation products, the metabolite prosulfocarb sulfoxide was qualitatively evaluated during the dissipation study. Traces of metabolite were detected in the S and S + GC (data not shown), of molecular ion $[M+H]^+$ of prosulfocarb sulfoxide at m/z of 267.4, and its fragmentation ion m/z at 128.10. Presence of traces of prosulfocarb sulfoxide has also been reported by Braun et al. (2017).

3.2. Mass balance of ^{14}C-prosulfocarb during its dissipation in unamended and amended soil

Fig. 2 includes the total ^{14}C-prosulfocarb mass balance corresponding to the extracted by $CaCl_2$ aqueous solution and methanol, mineralized, and non-extractable fractions of ^{14}C-prosulfocarb in both unamended and amended soils up to 62 days. The total mass balance (percentage of ^{14}C applied initially) was > 80% when the dissipation of ^{14}C-prosulfocarb was close to 50% in S and S + GC (Figs. 1 and 2). The total ^{14}C mass balance then decreased to 60%-70% at the end of the dissipation period under all conditions. A low mass balance has also been reported for other compounds, and it is usually attributed to herbicide volatilization, photodecomposition or biodegradation processes, recording balances of close to 100% when these processes do not occur (Merlin et al., 2016; Sukul et al., 2010; Wolters et al., 2003). The

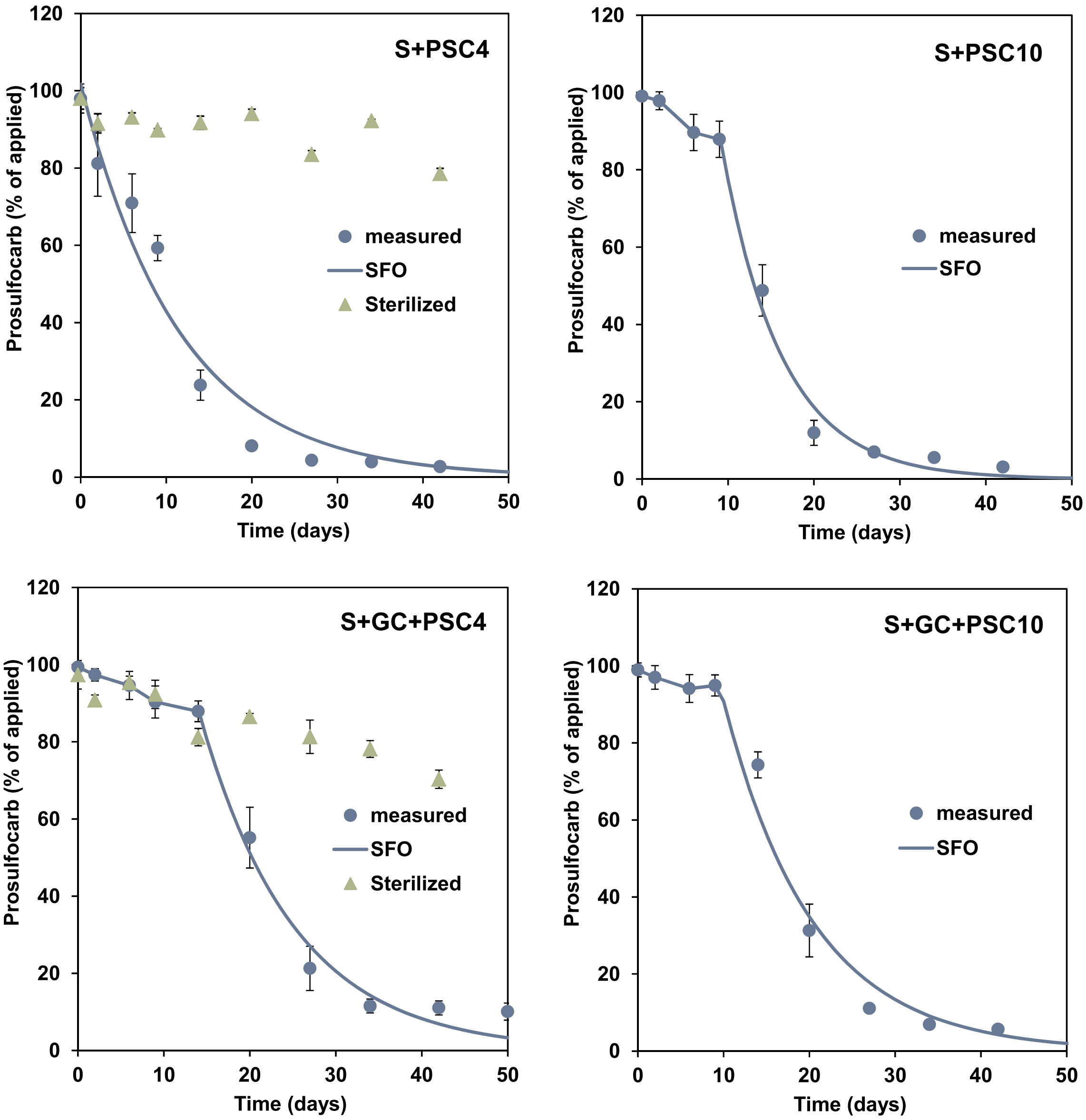

Fig. 1. Dissipation kinetics of non-labeled prosulfocarb (PSC) applied at two doses in unamended (S + PSC4 and S + PSC10) and GC-amended soils (S + GC + PSC4 and S + GC + PSC10). Error bars indicate the standard deviation of the mean value (n = 4).

mass balance results here indicate that the sum of water and organic extractable fractions accounts for the highest amounts up to the dissipation of ^{14}C-prosulfocarb close to 50%. The ^{14}C-herbicide was then mainly mineralized or formed non-extractable residues.

The amounts extracted with $CaCl_2$ aqueous solution, which constitute the more readily available herbicide fraction, ranged between 14% and 8% in S up to DT_{50} and then decreased to near 1% over time. These amounts in S + GC decreased below 5%, and less variability was observed in all the incubation times, reflecting the effect of GC in the water extractable fraction (Fig. 2). A similar effect has been reported for isoproturon in unamended soil and soil amended with biochar, although the effect of biochar is more evident for decreasing isoproturon

Table 2
Kinetic parameters for the dissipation of prosulfocarb (PSC) applied at low and high dose in unamended (S) and amended (S + GC) soils.

		SFO model				FOMC model				
Soil treatment	PSC dose (mg kg^{-1})	k (day^{-1})	DT_{50} ± SD (days)	r^2	χ^2	α	β	DT_{50} ± SD (days)	r^2	χ^2
S	4	0.086	8.0 ± 0.3d	0.961	13.8	1.79×10^5	2.08×10^6	8.1 ± 0.2d	0.961	15.7
S	10	0.142	13.9[a] ± 0.5c	0.993	11.0	1.04×10^4	6.77×10^4	13.5[a] ± 0.5c	0.993	12.9
S + GC	4	0.091	21.6[b] ± 1.3a	0.979	10.8	1.35×10^5	1.48×10^6	21.6[b] ± 1.4a	0.979	11.9
S + GC	10	0.096	16.2[a] ± 0.4b	0.968	13.6	6.53×10^4	6.73×10^5	16.1[a] ± 0.4b	0.967	15.0

Prosulfocarb dissipation curves were best fitted to SFO model (lower χ^2 values). DT_{50} values in the same column followed with the same letter are not significantly different ($P < 0.05$).

[a] DT_{50} value include a lag phase (slow dissipation) of 9 days.

[b] DT_{50} value include a lag phase (slow dissipation) of 14 days.

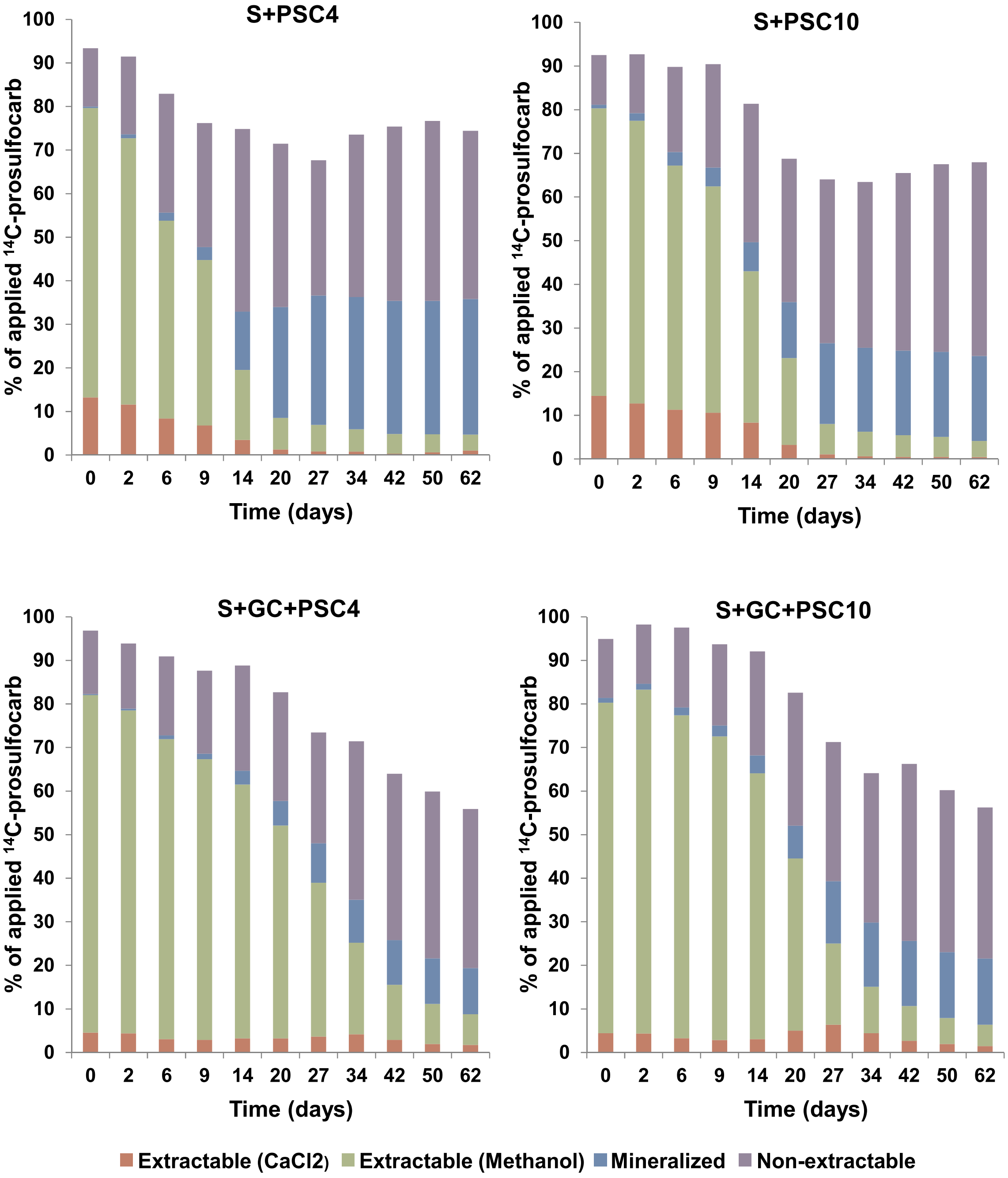

Fig. 2. $CaCl_2$-extractable, MeOH-extractable, mineralized and non-extractable fractions of ^{14}C-prosulfocarb applied at two doses in unamended (S + PSC4 and S + PSC10) and GC-amended soils (S + GC + PSC4 and S + GC + PSC10).

extractability, and an almost immediate sequestration of herbicide has been described (Reid et al., 2013). These results indicate higher herbicide bioavailability in the S due to lower sorption than in the amended ones regardless of the herbicide dose applied. The temporal decrease in extractable amounts in S and S + GC clearly showed the herbicide's aging effect over time as observed for other compounds (Álvarez-Martín et al., 2016).

The amounts of ^{14}C-prosulfocarb extracted with methanol were also high during the 50% dissipation of the compound. These amounts decreased in S during the rapid dissipation period, and were below 4% for both herbicide doses applied at the end of the incubation time. The percentages organically extracted in S + GC decreased to 7%-5% at the end of the incubation time (Fig. 2). The effect of the prosulfocarb dose applied was insignificant, as indicated previously for the water extractable fraction. The amount of ^{14}C-prosulfocarb residues extracted with methanol was slightly higher in amended soils than in unamended ones (Fig. 2), and they were significantly higher than those extracted with $CaCl_2$ aqueous solution, which was as expected given the higher solubility of prosulfocarb in organic solvents than in water (EFSA, 2007).

The decrease in extractability during incubation could occur when non-labeled degradation products (not determined) or non-extractable bound residues are formed. A similar pattern of decreasing amounts extracted with methanol has been found for other compounds such as metalaxyl or linuron (Marín-Benito et al., 2012, 2014). However, the variability of the methanol-extractable fraction was not observed for other compounds with lower hydrophobicity than prosulfocarb, such as penconazole, tebuconazole or indaziflam (Alonso et al., 2015; Álvarez-

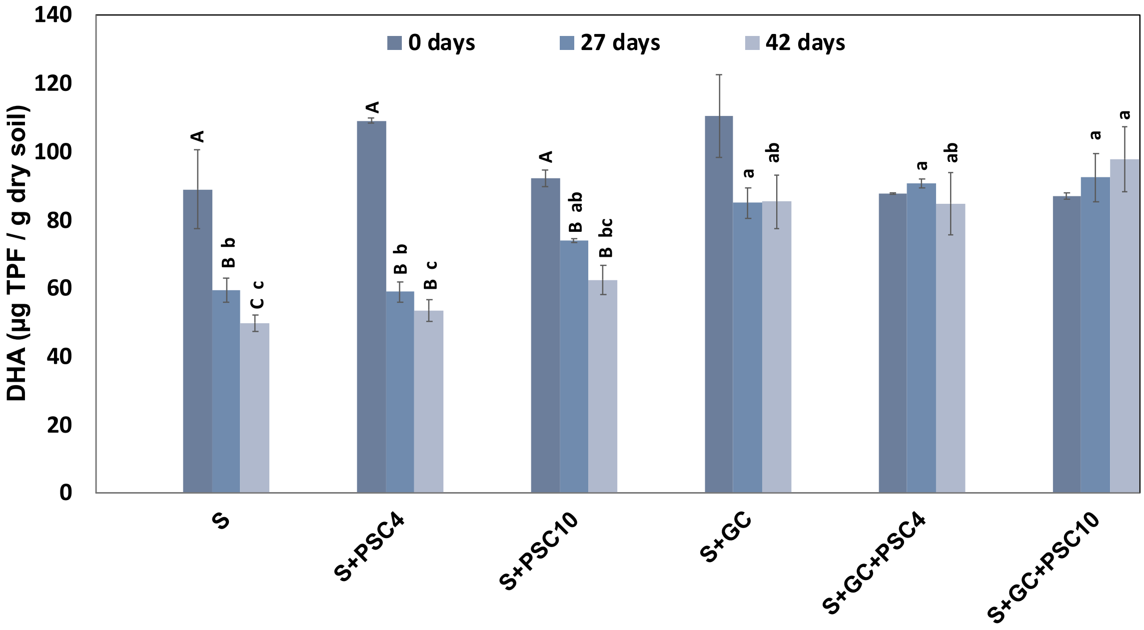

Fig. 3. Soil dehydrogenase activity (DHA) for unamended (S) and GC-amended soil (S + GC) untreated and treated with prosulfocarb at two doses (PSC4 and PSC10) at 0, 27 and 42 days. Bars indicate the standard deviation of the mean ($n = 4$). Different lowercase and uppercase letters indicate significant differences ($p \leq 0.05$) between treatments at the same sampling time and between sampling times within the same treatment, respectively. Lack of letters indicates no significant differences.

Martín et al., 2016; Marín-Benito et al., 2012).

The mineralization of prosulfocarb applied at low and high doses with the production of $^{14}CO_2$ increased rapidly during the incubation time, and percentages of 31%-20% in S and of 11%-15% in S + GC were reached over time (Fig. 2). This is consistent with the microbial pathway indicated for the dissipation of unlabeled prosulfocarb. Prosulfocarb mineralization increased slowly over incubation time because it is ^{14}C-labeled in the benzyl ring, and this group is less accessible for the mineralization of microorganisms with respect to other compounds ^{14}C-labeled in non-aromatic groups with rapid $^{14}CO_2$ evolution (Álvarez-Martín et al., 2016; Barriuso et al., 2008).

In a recent study, the mineralization of prosulfocarb was slower than that in the present study after 28 days of incubation (12.1%) in a soil with OC of 1.04% (Braun et al., 2017). However, the EFSA report indicates a mineralization percentage of 38% of ^{14}C-prosulfocarb in a soil with OC of 2.6% after 96 days (EFSA, 2007). The high percentage of prosulfocarb mineralized and the low percentage of ^{14}C-total balance previously indicated could be explained by prosulfocarb volatilization, which is a non-trapped fraction that is different to the $^{14}CO_2$ evolved from mineralization. Some thiocarbamates are reported to be volatile compounds (Ekler, 1988). Prosulfocarb is considered a slightly volatile (vapour pressure at 20°C (mPa) = 0.79; PPDB, 2018) compound, indicating that some losses due to volatilization are to be expected (EFSA, 2007; PPDB, 2018). Some studies have indicated losses of this herbicide from the soil by volatilization (Carlsen et al., 2006) or its detection in air or rainwater, especially in the autumn after its application (Kreuger et al., 2017), with prosulfocarb detection being higher in rainwater than in surface water samples from nearby agricultural streams draining treated fields.

^{14}C-prosulfocarb mineralization was higher in S than in S + GC, especially when the lower dose of herbicide was applied (S + PSC4) (Fig. 2). In general, a similar behaviour has been described for other pesticides (tebuconazole, linuron, metalaxyl, isoproturon and atrazine in unamended and amended soils (Álvarez-Martín et al., 2016; Jablonowski et al., 2013; Marín-Benito et al., 2012, 2014; Reid et al., 2013). These results reveal the influence organic amendment has on pesticide bioavailability to decrease its biodegradation. Abdelhafid et al. (2000) also report the decrease in the amount of pesticide mineralized in amended soils because of the use of the OM added with the amendment by the microorganisms instead of the herbicide. In addition, the lag phase observed initially in the prosulfocarb dissipation process in the S + GC could also delay its biodegradation due to the soil microbial community's prior period of adaptation.

The formation of non-extractable ^{14}C-residues was observed in S and S + GC and increased over time. In S there was a rapid formation of non-extractable residues of 11%-13%, increasing up to 30% during the 50% dissipation of herbicide, and the amounts still increased up to 39%-44% over time (Fig. 2). In S + GC, the rapidly formed non-extractable residues account for 14%-15%, and increased in a similar way to S, and were 35%-37% after 62 days of incubation (Fig. 2). These percentages were higher than that reported for the formation of non-extractable residues, which accounted for 27% after 96 days in an unamended soil (EFSA, 2007). However, the percentages of non-extractable residues in both unamended and amended soils could not be compared due to the lower total mass balance in the amended soil compared to the unamended one.

The pattern of formation of non-extractable residues is similar to that obtained for other pesticides. Increased amounts with incubation time have been found for different herbicides and fungicides (Álvarez-Martín et al., 2016; Botterweck et al., 2014; Jaquet et al., 2014; Marín-Benito et al., 2014). The degradation or mineralization of these non-extractable residues has been suggested when there is a re-equilibration between the sorbed and soluble phases (Alonso et al., 2015). In general, the formation of non-extractable residues is continuous to a greater or lesser extent as for other compounds (Alonso et al., 2015; Barriuso et al., 2008; Kästner et al., 2014; Marín-Benito et al., 2014). It has been pointed out that these non-extractable residues are immobilized in soils by increasing the physicochemical interactions between the compound and the soil components, especially OM and humic substances, over the incubation period, and they could not be extracted without altering their chemical structure (Burauel and Führ, 2000; Merlin et al., 2016).

3.3. Soil dehydrogenase activity during prosulfocarb dissipation

Fig. 3 shows the soil DHA trends in S and S + GC during the prosulfocarb dissipation experiment. Non-significant differences were

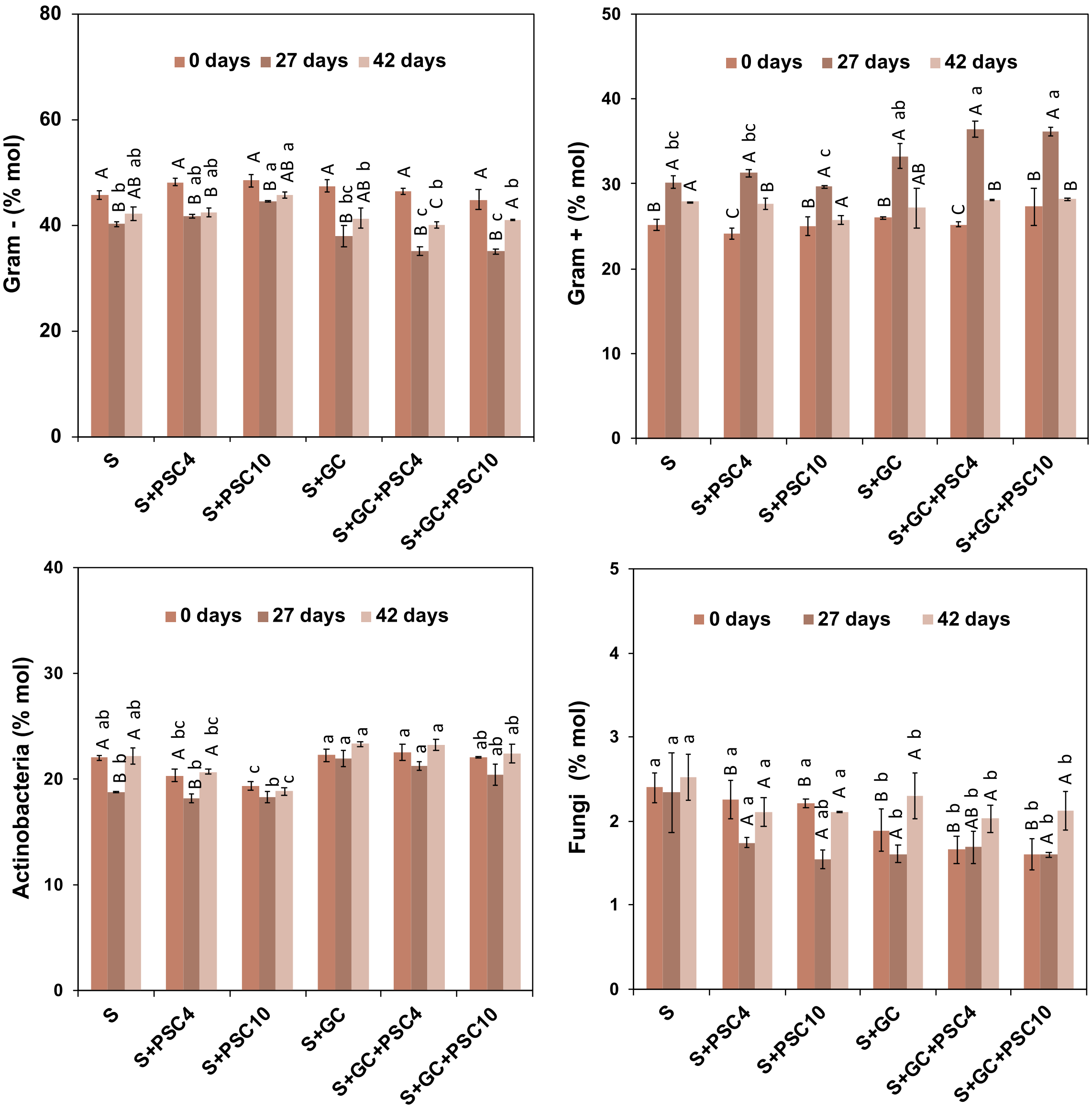

Fig. 4. Relative abundance (% mol) of PLFAs specifically diagnostic of Gram-negative and Gram-positive bacteria, *Actinobacteria* and fungi in unamended (S) and GC-amended soil (S + GC) untreated and treated with prosulfocarb at two doses (PSC4 and PSC10) at 0, 27 and 42 days. Bars indicate the standard deviation of the mean (n = 4). Different lowercase and uppercase letters indicate significant differences ($p \leq 0.05$) between treatments at the same sampling time and between sampling times within the same treatment, respectively. Lack of letters indicates no significant differences.

initially found between S or S + GC soils treated with low or high doses of prosulfocarb and the corresponding non treated S or S + GC soils. The application of GC significantly enhanced the soil DHA at 27 and 42 days, while the DHA in S decreased from initial time up to 42 days. Prosulfocarb could stimulate the microbial activity in S + GC over time due to a higher percentage of herbicide in the $CaCl_2$ aqueous + methanol extractable fraction than in S, being bioavailable for microorganisms longer to be used as a carbon and energy source.

The lack of negative effects of prosulfocarb on DHA agrees with prior research in which prosulfocarb and other thiocarbamate herbicides do not have a significant negative impact on soil microbial activity (Das et al., 2015; García-Delgado et al., 2018; Saison et al., 2009) or even increase the growth and activity of soil microorganisms (Bhowmick et al., 2014). The high DHA of unamended soils at initial time can be related to the rewetting process that increases soil respiration and the metabolic quotient with a rapid rise in microbial biomass (Sun et al., 2017). Unamended soils decrease DHA over time because microbial biomass consumes labile carbon, and the extractable fraction of prosulfocarb decreases rapidly after the first sampling time. However, GC-amended soils contain higher amounts of nutrients that maintain high microbial activity for a long time. García-Delgado et al. (2018) have obtained similar results at field scale.

3.4. Analysis of the phospholipid fatty acid profile over prosulfocarb dissipation in unamended and GC-amended soils

The relative abundance of PLFAs that diagnose Gram-negative bacteria, Gram-positive bacteria, *Actinobacteria* and fungi in S and S + GC was evaluated at 0, 27 and 42 days after prosulfocarb application (Fig. 4). Initially, no significant differences were observed in the relative abundance of Gram-positive and Gram-negative bacteria or fungi. However, the relative abundance of *Actinobacteria* was significantly higher in S + GC + PSC4 and S + GC + PSC10 than in S + PSC4 and S + PSC10, respectively, but no significant differences were observed between S and S + GC (control soils). The GC amendment buffered the initial negative effect of prosulfocarb on the relative

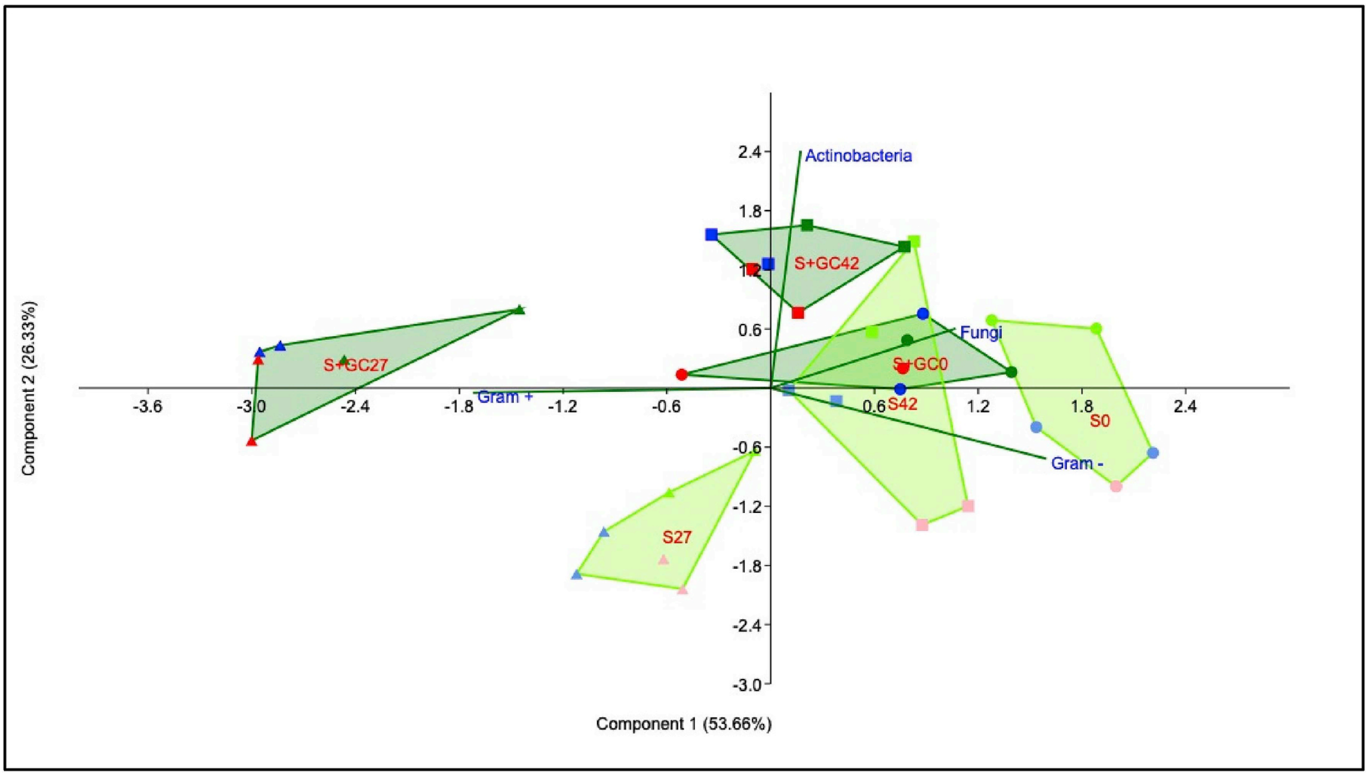

Fig. 5. Principal component analysis (PCA) showing loading scores for Gram-negative and Gram-positive bacteria, *Actinobacteria*, fungi, soil DHA, soil humidity, and percentage of remaining prosulfocarb and sampling time and scores of each treatment (control: green; low dose: blue; high dose: red) and sampling time (0 days: circles; 27 days: triangles; 42 days: squares) on the two main components. Unamended (S) and GC-amended (S + GC) soils were denoted by light and dark colors, respectively. Percent variability explained by each principal component is shown in parentheses after each axis legend. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article)

abundance of *Actinobacteria,* probably because of the lower percentage of prosulfocarb extractable with aqueous $CaCl_2$ in S + GC soils than in unamended soils (Fig. 2).

At 27 days after herbicide application, prosulfocarb seems to promote Gram-negative bacteria in S (the highest abundance was observed in S + PSC10 compared to the untreated soil), but this effect was buffered in the case of S + GC, and no significant differences were found. The relative abundance of Gram-positive bacteria, *Actinobacteria* and fungi was not significantly different between the control and low or high dose of prosulfocarb in S and S + GC, respectively. However, Gram-positive bacteria and *Actinobacteria* were positively affected by the GC, which increased their relative abundances.

At the end of the assay (42 days), prosulfocarb did not have any significant effects on the relative abundance of Gram-negative and Gram-positive bacteria and fungi, irrespective of the treatment or use of GC. Prosulfocarb applied at 10 mg kg^{-1} decreases the relative abundance of *Actinobacteria* solely in the case of S. This effect was buffered again in the case of GC-amended soil.

Gram-negative bacteria behaved in the opposite way to Gram-positive bacteria during the assay. This pattern has previously been reported by García-Delgado et al. (2019) under field conditions in GC-amended soils. Because of the lack of significant differences between soils untreated and treated with prosulfocarb, the shift of Gram-negative and Gram-positive bacteria was not caused by prosulfocarb. The relative abundances of *Actinobacteria* and fungi did not record significant changes at the end of the assay in S and S + GC. There was therefore little disturbance of the microbial structure over the prosulfocarb degradation process, and the herbicide was not responsible for this change.

The Pearson correlation coefficients between prosulfocarb residue, soil moisture content, soil microbial structure and DHA (Table S1) and the principal components analysis (PCA) of the soil microbial structure (Fig. 5) were determined to evaluate the relationship between these variables. The relative percentage of Gram-positive bacteria was negatively correlated with the relative percentage of Gram–negative bacteria and fungi (Table S1, Fig. 5). The negative or positive correlations observed between the prosulfocarb residue and the percentage of different microbial groups (Table S1) could indicate that the herbicide favours the relative population of Gram-negative bacteria versus Gram-positive bacteria and *Actinobacteria*. However, this effect seems unlikely because of the lack of significant differences between untreated soil and soil treated with prosulfocarb (Fig. 4), and it may be related to the evolution of the microbial structure and the dissipation of prosulfocarb over time. On the other hand, the positive or negative correlations between soil moisture content and the relative abundance of different microbial groups and prosulfocarb residue (Table S1) could derive from the input of GC that increased the water-holding capacity of amended soils, giving rise to positive and negative effects on these parameters.

The evolution of soil microbial community during the assay revealed that soil microbial community in S and S + GC soils was initially similar, which was related to a high relative population of Gram-negative bacteria and fungi (Fig. 5). Likewise, the proximity of untreated S and S + GC and these soils treated with the herbicide at 4 and 10 mg kg^{-1}, respectively, confirmed the low impact of prosulfocarb in soil microbial structure during the assay. In fact, PERMANOVA analysis did not find any significant differences between untreated and treated soils. As reported above, PERMANOVA analysis revealed significant differences between S and S + GC soils ($p < 0.01$). Similar results have been reported at field scale, where soil microbial structure was clearly influenced by the application of GC, but no differences were found between untreated soils and soils treated with prosulfocarb (García-Delgado et al., 2018).

At 27 days after prosulfocarb application, the microbial structure of S and S + GC soils shifted towards Gram-positive bacteria. However, S + GC (irrespective of prosulfocarb application or doses) was more closely related to Gram-positive bacteria and *Actinobacteria*, and S was more closely related to Gram-negative bacteria than S + GC (Fig. 5). At the end of the assay (42 days), the microbial structures of S and S + GC shifted back nearer to their initial state, more closely related to Gram-negative bacteria, fungi and *Actinobacteria* than the microbial structure at 27 days.

The low impact of prosulfocarb on soil microbial structure could be related to the dynamic of prosulfocarb in soil, mainly to its high hydrophobicity, fast dissipation and volatile nature, which minimized prosulfocarb availability to soil microorganisms (Braun et al., 2017; García-Delgado et al., 2018; Nunes et al., 2013) as shown in Figs. 1 and 2. Other thiocarbamate herbicides such as thiobencarb had no toxic effects on soil microbiota population or activity (Bhowmick et al., 2014; Das et al., 2015; Saison et al., 2009). Therefore, according to our findings and the literature, prosulfocarb and other thiocarbamate herbicides could be recommendable herbicides for controlling weeds because of their fast degradation and low toxicity towards soil microbiology.

4. Conclusions

The herbicide prosulfocarb recorded faster dissipation and higher bioavailability in the unamended soil than in the GC amended soil due to its lower sorption regardless of the dose of herbicide applied. A lag phase was observed initially in the prosulfocarb dissipation process in the GC-amended soil that delays its biodegradation. A temporal decrease in extractable amounts was observed in unamended and amended soil, and revealed herbicide aging over time. However, prosulfocarb mineralization and non-extractable residues increased slowly over incubation time in both unamended and amended soils. The total ^{14}C mass balance was in the range 60%-70% under all conditions at the end of the dissipation period, indicating that part of the herbicide was lost through volatilization or other processes such as incorporation of metabolites in microbial biomass, although this should be confirmed with further studies.

The results indicate that prosulfocarb stimulated soil DHA in amended soil over time due to a higher percentage of herbicide present in the extractable fraction than in unamended soil. No negative effects of prosulfocarb on DHA were observed, and PFLA results indicate the low impact of prosulfocarb in the soil microbial structure during the assay, although this structure was clearly influenced by the application of GC. These results reveal the influence organic amendment has on herbicide bioavailability to decrease its biodegradation and buffer its impact on the soil microbial structure. Based in the present laboratory and previous field studies results, prosulfocarb presents rapid dissipation, although the non-extractable fraction of the herbicide may persist over time, and low impact on soil microbiology. Further studies including different soils and amendments would be necessary to increase the knowledge about the effect of prosulfocarb on soil microbial communities.

Conflicts of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoenv.2019.109395.

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Material Suplementario

SUPPLEMENTARY MATERIAL

ASSESSMENT OF ^{14}C-PROSULFOCARB DISSIPATION MECHANISM IN SOIL AFTER AMENDMENT AND ITS IMPACT ON THE MICROBIAL COMMUNITY

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Table S1.

Pearson correlation coefficients between relative percentage of Gram-negative and Gram-positive bacteria, *Actinobacteria* and fungi, percentage of remaining prosulfocarb (PSC), soil moisture, soil dehydrogenase activity (DHA) and ratios Gram+/Gram- bacteria, total Gram+ (Gram-positive + *Actinobacteria*)/Gram- and total bacteria/fungi. Significant correlations were denoted by asterisks and bold font.

	Gram-	Gram+	*Actinobacteria*	Fungi	PSC	Moisture	DHA	Gram+/Gram-	Total +/Gram-	Bacteria/Fungi
Gram-	1									
Gram+	**-0.910*****	1								
Actinobacteria	-0.099	-0.156	1							
Fungi	0.262	**-0.462****	0.082	1						
PSC	**0.454****	**-0.335***	-0.003	-0.143	1					
Moisture	**-0.451****	**0.368***	**0.664*****	**-0.417***	0.037	1				
DHA	0.161	-0.108	**0.377***	-0.177	0.242	**0.561*****	1			
Gram+/Gram-	**-0.945*****	**0.973*****	-0.073	-0.356	**-0.393***	**0.399***	-0.076	1		
Total +/Gram-	**-0.969*****	**0.925*****	0.148	-0.312	**-0.410***	**0.530*****	-0.013	**0.973*****	1	
Bacteria/Fungi	-0.277	**0.496****	-0.080	**-0.968*****	0.046	**0.404***	0.195	**0.400***	**0.355***	1

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

CAPÍTULO 6

Resumen

CAPÍTULO 6: Transport of ^{14}C-prosulfocarb through soil columns under different amendment, irrigation and incubation regimes

RESUMEN

La aplicación de enmiendas orgánicas al suelo es una práctica común en agricultura para aumentar el contenido de nutrientes y de materia orgánica (MO). Esta práctica puede impedir la posible contaminación de agua por pesticidas debido a la modificación de su biodisponibilidad o de su retención por el suelo. Los estudios previos sobre la disipación y movilidad del prosulfocarb en un suelo con dos enmiendas de compost vegetal (GC) diferentes y diferentes regímenes de riego en condiciones de campo indicaron un aumento de la persistencia del herbicida en el suelo enmendado y la influencia del contenido de agua del suelo y de carbono orgánico disuelto (COD) en la movilidad de prosulfocarb a través del perfil del suelo. Con el fin de ampliar nuestro conocimiento sobre la influencia de estos factores en la movilidad de prosulfocarb en el suelo cuando se aplica simultáneamente con el GC, se llevó a cabo este estudio para evaluar el efecto de diferentes flujos de agua en la movilidad del herbicida prosulfocarb sometido a diferentes estados de envejecimiento en el suelo.

En el experimento de laboratorio se utilizaron columnas empaquetadas con suelo sin enmendar y enmendado con GC (20% en peso seco). El herbicida se añadió marcado en ^{14}C con una concentración de 10 mg kg^{-1} (2.5 veces la dosis agronómica) y una actividad de 10 kBq mL^{-1}. Se evaluó el lavado del herbicida después de añadir un volumen de agua (0.1M de $CaCl_2$) de 500 mL de forma continua con una velocidad de flujo de 1 mL min^{-1} (flujo saturado) o de forma discontinua (20 mL diarios durante 25 días) (flujo saturado-no saturado). El lavado de las columnas comenzó un día después de añadir el herbicida a la superficie de éstas o después de un periodo de envejecimiento del herbicida en el suelo de 28 días en oscuridad a 20 ºC. Se determinaron las fracciones lixiviada, mineralizada y retenida en cada columna. El ion cloruro (KCl) se utilizó como ion trazador y la movilidad de este ion se estudió aplicando los mismos flujos de lavado que en las columnas tratadas con herbicida.

La cantidad total de prosulfocarb lixiviada en las columnas de suelo sin enmendar fue del 13.0% y 26.7% de la cantidad añadida inicialmente para el flujo saturado y saturado-no saturado, respectivamente. Estos valores fueron similares bajo flujo saturado (12.2%) y ligeramente mayores bajo flujo saturado-no saturado (32.5%) en el suelo

enmendado. Cuando el herbicida fue incubado en las columnas, las cantidades lixiviadas disminuyeron 2.1 y 1.9 veces en el suelo sin enmendar, y 2.9 y 1.6 veces en el suelo enmendado bajo flujo saturado y saturado-no saturado, respectivamente. Las pérdidas del herbicida durante el proceso de envejecimiento o procesos de adsorción del herbicida dependientes del tiempo podrían explicar estos resultados.

Por otra parte, las mayores cantidades de prosulfocarb retenidas en las columnas de suelo enmendado (77.4%-85.2%) que en las columnas de suelo sin enmendar (57.1%-60.8%) bajo flujo saturado pusieron de manifiesto una fuerte interacción entre el herbicida y el suelo enmendado. Este comportamiento no se observó bajo flujo saturado-no saturado, donde las cantidades retenidas fueron similares a las determinadas en suelos sin enmendar. Una cinética de lavado más lenta de prosulfocarb podría tener lugar en ambos suelos bajo flujo saturado-no saturado favoreciendo posiblemente la formación de productos de degradación con capacidad de lixiviación más elevada. La distribución del herbicida en las columnas indicó la mayor retención en el primer tramo en todas las condiciones estudiadas.

Las cantidades de herbicida mineralizado fueron bajas en columnas no incubadas ($\leq 0.40\%$) para los suelos enmendados y sin enmendar bajo flujo saturado. Sin embargo, estas cantidades mineralizadas aumentaron hasta 19 veces bajo flujo saturado en las columnas incubadas en comparación con las columnas no incubadas. En las columnas bajo flujo saturado-no saturado las cantidades lixiviadas no se vieron afectadas por la incubación del herbicida.

Los balances totales de ^{14}C fueron > 73% y > 80% en las columnas de suelo sin enmendar y enmendado con GC, respectivamente. La posible pérdida de herbicida por volatilización se observó en columnas de suelo sin enmendar tras su incubación, y en menor extensión en columnas de suelo enmendado.

Los resultados obtenidos demostraron la importancia de diversos factores (la enmienda orgánica, el flujo de agua y el envejecimiento de los herbicidas) en el control de la lixiviación del herbicida prosulfocarb a través del perfil del suelo y completan la información obtenida de los estudios de campo realizados anteriormente.

Artículo

Transport of ^{14}C-prosulfocarb through soil columns under different amendment, herbicide incubation and irrigation regimes

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Transport of ^{14}C-prosulfocarb through soil columns under different amendment, herbicide incubation and irrigation regimes

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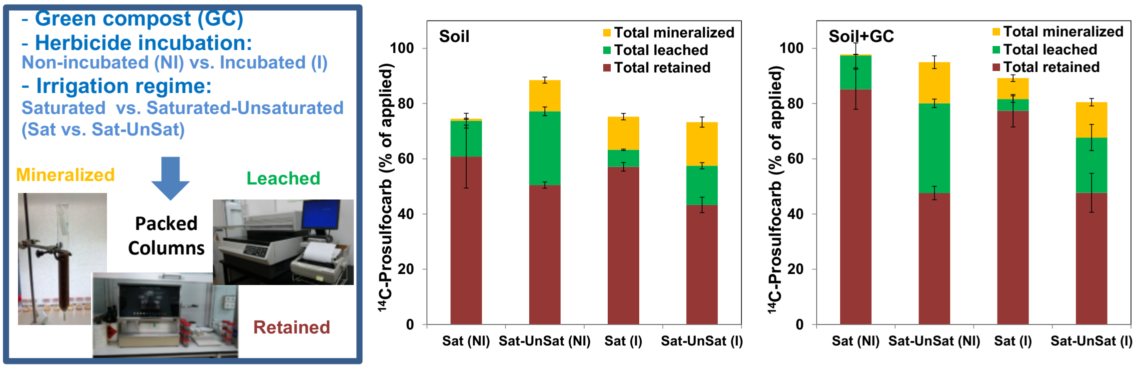
Highlights

- Leaching of prosulfocarb in green compost-amended soil (S+GC) columns was studied
- The leached amounts decreased when the herbicide was incubated for 28 days in S and S+GC
- The total retained amounts were lower in S than in S+GC columns under saturated flow
- Prosulfocarb was retained in the first segment of S and S+GC columns under all conditions
- Herbicide incubation notably increased the mineralized amount under saturated flow

Graphical Abstract

Effect of Different Factors on the Leaching of ^{14}C-Prosulfocarb in Soil



Abstract

This study sets out to evaluate the effect on the leaching of prosulfocarb through packed soil columns of applying green compost (GC) as an organic amendment (20% w/w), herbicide ageing over 28 days in the soil (incubation vs. no incubation), and two different irrigation regimes (saturated or saturated-unsaturated flows). Peak concentrations decreased after herbicide incubation in the columns for both unamended (S) and amended (S+GC) soils under both flow regimes. The leached amounts decreased when the herbicide was incubated for 28 days in S (2.1 and 1.9 times) and S+GC (2.9 and 1.6 times), under saturated or saturated-unsaturated flow, respectively. In the S columns, the total amounts retained (43.3%-60.8%) were lower than the ones obtained for the S+GC columns under saturated flow (77.4%-85.2%), suggesting a stronger interaction between the herbicide and the GC-amended soil. This behaviour was not observed under saturated-unsaturated flow, as the total amounts retained were similar in both the S and S+GC columns. Prosulfocarb was primarily retained in the first segment of the S (> 28%) and S+GC (> 43%) columns under all conditions. Incubation time did not greatly affect the herbicide retention, but it significantly increased the mineralized amount under saturated flow. The total balances of ^{14}C-prosulfocarb were > 73% and > 80% in the S and S+GC columns, respectively, indicating that amendment decreased prosulfocarb loss by volatilization. Several factors, such as amendment, herbicide ageing and water flow, proved to be important for controlling the leaching of this herbicide through the soil profile.

Keywords

Prosulfocarb, soil, green compost, leaching, water saturation, ageing

1. Introduction

The application of pesticides on different crops to increase yields may lead to the release of pollutants and residues into waters and soils (Climent et al., 2019; Herrero-Hernández et al., 2016; Pose-Juan et al., 2015). Prosulfocarb (S-(phenylmethyl)-dipropylcarbamothioate) is a thiocarbamate herbicide used at pre- and post-emergence for weed control in different crops, such as potatoes or winter cereals. It is a hydrophobic herbicide with low water solubility, a Kf_{oc} of 1367-2339 mL g^{-1}, and a GUS index of 0.84, indicating high sorption and low leachability in soil (EFSA, 2007; PPDB; 2019). Prosulfocarb could reach surface water after its application to different agricultural soils through point sources (Neumann et al., 2003), spray drift (Arts et al., 2006), recycled water (Busetti et al., 2015), and rainwater contamination (Bernhardt et al., 2004). However, to our knowledge, there are no studies on prosulfocarb's potential to reach groundwater.

Our previous studies have reported the dissipation and mobility of prosulfocarb in a soil with two different green compost (GC) amendments and different irrigation regimes under field conditions (Marín-Benito et al., 2018a,b). The simultaneous application of GC increases the herbicide's persistence in the soil. Prosulfocarb's leaching through the soil profile was influenced mainly by soil water content and dissolved organic carbon (DOC) content. A low amount of water in the soil means the herbicide is less mobile toward deeper soil layers. By contrast, the effect of the DOC of GC-amended soil could favour herbicide mobility (Marín-Benito et al., 2018b). We therefore need to further our knowledge of the influence these factors have on the leaching of prosulfocarb in organically-amended soils. In a previous laboratory study, the herbicide prosulfocarb recorded faster dissipation and higher bioavailability in the unamended soil than in the GC amended soil due to its lower sorption regardless of the dose of herbicide applied (Barba et al., 2019).

The application of organic amendments to soils is a common agricultural practice used to increase nutrient content and organic carbon (OC). This practice could prevent the possible contamination of groundwater by pesticides due to the modification of their bioavailability and retention by the soil (Marín-Benito et al., 2018c; Pose-Juan et al., 2018). Organic residues are characterized by high OC content, which is one of the main factors influencing the adsorption process in soils. Organic amendments could act as a sorbent to prevent the spread or leaching of pesticides through the soil profile (Álvarez-Martín et al., 2016; Marín-Benito et al., 2013, 2018c). Leaching may be reduced by structural changes in the porosity induced by the change in OC content (Worrall et al., 2001). In addition, high levels of dissolved organic matter (DOM) could cause the formation of complexes with pesticides, due to their adsorption by the DOC in solution, increasing their mobility to deeper soil layers (García-Jaramillo et al., 2014; Marín-Benito et al., 2009; Thevenot et al., 2009).

Leaching studies have been carried out in columns of soils amended with olive mill waste (Aharonov-Nadborny et al., 2016), spent mushroom substrate (SMS) (Álvarez-Martín et al., 2017), sheep manure, spent coffee grounds, composted pine bark, and coir (Fenoll et al., 2014), biochar (Gámiz et al., 2017), manure and fly ash (Majumdar and Singh, 2007), and grape marc, pine wastes, urban solid wastes, sewage sludge, and SMS (Marín-Benito et al., 2018c). These studies have reported a reduction in pesticide leaching due to enhanced pesticide sorption in organically-amended soils. Leaching can be decreased due to the pesticide's retention by the solid organic matter (OM) of the amendments (Majumdar and Singh, 2007), although simultaneously leaching can also increase by the DOC from the OM of organic amendments.

Mobility studies in soil amended with organic residues have been carried out under saturated or saturated-unsaturated flow regimes, but studies under both flow conditions are rare (Álvarez-Martín et al., 2017). Experiments conducted under a saturated flow condition simulate the worst-case scenario (Cederlund et al., 2017; Ghosh and Singh, 2012, Larsbo et al., 2013), while experiments carried out under a saturated-unsaturated flow condition are closer to real field conditions, as they simulate rainfall and irrigation events (Aharonov-Nadborny et al., 2016; Fenoll et al., 2014).

Leaching studies have usually been conducted without pesticide ageing in the amended soil (Gámiz et al., 2017; Majumdar and Singh, 2007). Leaching pesticides under different ageing states in unamended and amended soils is relevant because this factor and the flow regime largely determine the transport of these compounds and the potential risk of groundwater contamination (Álvarez-Martín et al., 2017; Milan et al., 2015). There is evidence to show that the timing of rainfall or irrigation relative to the time of pesticide application may be the major factor controlling leaching losses (Walker et al., 2005). The incubation of a soil-amendment-pesticide system could transform soil OM, altering the way pesticides interact with amended soils (Marín-Benito et al., 2012).

Because the lack of knowledge about prosulfocarb mobility through soil profile taking into account the factors pointed above, it is necessary to study the influence of the application of organic amendments to soil on this process under different conditions of herbicide incubation in the soil and irrigation. Results from this study will be relevant to complete our knowledge about the fate of prosulfocarb in the soil environment.

This paper's objective was to study the leaching of the herbicide prosulfocarb through packed soil columns. The effect of the following factors was evaluated: 1) the application of GC as an organic amendment to soil; 2) the incubation time following herbicide application to the soil; soil column washing started at Day 1 (non-incubated) or 28 days (incubated) after herbicide application; and 3) the irrigation regime applied; saturated with continuous irrigation or saturated-unsaturated with intermittent irrigation.

2. Materials and methods

2.1. Chemicals

A non-labelled prosulfocarb analytical standard (PESTANAL®, > 98.9% purity) was supplied by Sigma-Aldrich Química S.A. (Madrid, Spain). The labelled [Ring-U-^{14}C] prosulfocarb (specific activity 3.16 MBq mg^{-1}, 94.8% purity) was supplied by IZOTOP (Budapest, Hungary). Prosulfocarb has a water solubility of 13.2 mg L^{-1} (20ºC), and a log K_{ow} of 4.48 (pH 7, 20ºC) (PPDB, 2019).

2.2. Organic amendment

The green compost (GC) came from the pruning of plants and trees in gardens and parks, and it was supplied by the nursery El Arca (Salamanca, Spain). Its characteristics were determined in samples that were first homogenized and sieved (< 2 mm) (Barba et al., 2019). The GC's characteristics (on a dry weight basis) were as follows: pH 7.20, total OC content 24.1%, DOC content 0.703%; total N content 1.11%, C/N ratio 21.8, ash percentage 54.0%, and moisture content 48.6% (**Table S1**).

2.3. Unamended and amended soil characterization

The soil used in this study was classified as a Typic Haploxerept with sandy clay loam texture (57.63% sand, 16.97% silt, 24.98% clay, and 0.21% carbonate content). It was taken from the surface horizon (0-30 cm) at the Muñovela experimental farm (40º55'56"N latitude and 5º52'53"W longitude) belonging to the Institute of Natural Resources and Agrobiology of Salamanca (IRNASA-CSIC).

The amended soil was prepared by uniformly mixing soil with GC (S+GC) at a rate of 20% w/w (180 t ha^{-1}) on a dry weight basis. The soil and GC were mixed after sieving (< 2 mm), and its characteristics were determined (Barba et al., 2019). The pH, total OC, N content, and DOC were 7.35, 1.30%, 0.12% and 0.006%, respectively, in the unamended soil (S), and 7.30, 4.66%, 0.42% and 0.027% in the GC-amended soil (S+GC), also respectively (**Table S1**).

2.4. Soil column setup and leaching studies

Four herbicide leaching experiments were performed, following OECD guidelines (OECD, 2007), in triplicate for each soil treatment in glass columns of 3 cm (i.d.) x 25 cm (length) packed with 100 g of unamended or GC-amended soils (**Table S2**). These columns were oversaturated with distilled water to their maximum water-holding capacity, and then allowed to drain off the excess water for 24 hours, so the humidity conditions were the same as field capacity. The pore volume (PV) of the packed columns was estimated by the difference in weight between the water-saturated columns and dry columns (**Table 1**). Prosulfocarb was evenly applied by adding 1 mL of a 1000 µg mL^{-1} prosulfocarb solution in methanol to the top of the columns for a concentration of 10 mg kg^{-1} (2.5 times the agronomic dose), and an activity of approximately 10 kBq mL^{-1}. This concentration was similar to the concentration applied in the field (Marín-Benito et al., 2018b) to simulate the worst case scenario of these compounds leaching in soils (Khorram et al., 2015).

Leaching was carried out one day after herbicide application (non-incubated columns, NI) or after incubating the herbicide at 20ºC in the dark for 28 days (incubated columns, I) (**Table S2**). This incubation period was selected taking into account the relatively short half-life of prosulfocarb (Barba et al., 2019). A $CaCl_2$ aqueous solution (0.01M) was used instead of water to wash the columns to minimize the disruption of the soil mineral balance. Two different washing flow regimes were studied: saturated and saturated-unsaturated (**Table S2**). Under saturated flow regime, 500 mL of a 0.01M $CaCl_2$ solution were pumped continuously at a rate of 1 mL min^{-1}, always keeping the top of the column in contact with the water. Under the saturated-unsaturated flow regime, the same volume of $CaCl_2$ solution was added for 25 days (20 mL per day). Herbicide mineralization was measured over incubation time in the column, or when a saturated-unsaturated flow was applied. A ^{14}C trap consisting of a scintillation vial containing 1M NaOH (1 mL) was attached to the top of the column via a stainless-steel clip (Reid et al., 2002), and $^{14}CO_2$ from mineralized ^{14}C-prosulfocarb was periodically determined.

A Gilson MINIPULS 3 peristaltic pump (Gilson Inc. Middleton, WI, USA) was used to maintain the leaching flow constant. Fractions of leaching solution (20 mL) were collected by a Gilson F203 automated fraction collector. Once the leaching had been completed, the columns were cut into three segments (0-5, 5-10 and 10-15 cm), and the soil contained in each segment was dried and weighed.

2.5. Analysis of ^{14}C-prosulfocarb residues

The radioactivity of the leached fractions was measured in disintegrations per minute (dpm) on a Beckman LS 6500 liquid scintillation counter (Beckman Instruments Inc., Fullerton, CA). These measurements were determined in duplicate by mixing 1 mL of extract with 4 mL of scintillation cocktail (Ecoscint^{TM} A, National Diagnostics, Atlanta, GA). The dpm values recorded were compared to the dpm value obtained for the prosulfocarb standard solution. The quantification limit for the ^{14}C-prosulfocarb was determined as the background radioactivity (18.2-23.1 dpm) in the leached $CaCl_2$ solution. Determinations were carried out in duplicate for all the solutions, and the range of the coefficient of variation was always between 0.1% and 2%. $^{14}CO_2$ from mineralized ^{14}C-prosulfocarb trapped in the scintillation vial containing 1M NaOH (1 mL) was determined by adding 4 mL of scintillation cocktail, as previously indicated.

The remaining ^{14}C-prosulfocarb retained in the soil after leaching was determined by the combustion of triplicate 1 g samples of dried soil using a Biological Oxidizer (RJ. Harvey OX-500 Instrument Corporation, NJ) under O_2 excess at 900ºC. The $^{14}CO_2$ generated was trapped in a mixture of ethanolamine (1 mL) and scintillation cocktail (Oxysolve C-400, Zinsser Analytic, Berkshire, UK, 15 mL), and determined as indicated before. The oxidizer´s efficiency was calculated prior to sample combustion as the ratio between ^{14}C-standard activity applied in an inert material (mannitol) after combustion in an oven and ^{14}C-standard activity without combustion. ^{14}C-prosulfocarb recovery was always > 98%.

2.6. *Chloride ion leaching in soil columns*

Chloride ion (KCl) was used as an ion tracer to describe the dispersive characteristics of the columns used for herbicide leaching. An amount of 47 mg of chloride ion was applied per column (1 mL of a 100 g L^{-1} KCl aqueous solution). Chloride ion leaching was carried out with similar water flow regimes to those used in herbicide leaching (saturated flow and saturated-unsaturated flow). Chloride ion concentrations were determined using a Metrohm Ion Chromatograph (Metrohm Ltd., Switzerland) with a conductivity detector, following the method described by Rodríguez-Cruz et al. (2011).

2.7. *Data analysis*

The retardation factors, R, were determined as indicators of the peak shifts of the BTC for herbicide leaching relative to the chloride tracer in the unamended and amended soil columns. These factors were estimated according to the expression $R = 1 + K \rho / \theta$ (Marín-Benito et al., 2013), assuming that sorption–desorption isotherms are linear and reversible. In this expression, ρ is the bulk density of the soil (g cm^{-3}), θ is the volumetric water content or PV in the packed column divided by the total volume (cm^3 cm^{-3}), and K is the distribution coefficient for the linear adsorption of herbicide by soil (mL g^{-1}). K values were determined from the linearized adsorption isotherms obtained by the batch equilibrium technique for prosulfocarb with the soils used here (unpublished results). The parameters of the soil columns and the calculated values of adsorption constants (K) and retardation factors (R) are included in **Table 1**.

Standard deviation (SD) was used to indicate the variability in the leached, retained, or mineralized amounts of herbicide among replicates. Retained, leached, mineralized and total amounts of ^{14}C-prosulfocarb were subjected to an analysis of variance (ANOVA) to measure the effects of herbicide incubation and irrigation. IBM SPSS statistics v24 software package was used (SPSS Inc. Chicago, USA).

3. Results and discussion

3.1. Chloride ion leaching in soil columns

The chloride ion is widely used as a water flow tracer in soil columns because it is a conservative ion that is not retained or degraded in soils. The chloride breakthrough curves (BTC) were obtained for both unamended and amended soils (**Fig. 1**). They were symmetric, and maximum leaching occurred at about 1 PV, as expected in the percolation of conservative ions. An amount of chloride ion close to 100% of the ion applied was recovered at the end of the leaching process. This indicates that water flow is uniform in the columns used; tracer ion leaching is not affected by the water flow regime applied, and there are no preferential flows throughout the soil columns. The peak concentration recorded for the amended soil decreased with respect to the unamended soil (38% and 47%, respectively). A similar reduction was also observed in soils amended with other organic residues (Álvarez-Martín et al., 2017).

3.2. Breakthrough curves of prosulfocarb in unamended and amended soil columns

Experimental BTCs were obtained corresponding to prosulfocarb leaching in the unamended soil columns (**Fig. 1A,B**) and in the GC-amended soil columns (**Fig. 1C,D**) under two different herbicide incubation times (one day and 28 days of prosulfocarb ageing in soil before leaching) and flow regimes (saturated and saturated-unsaturated).

In general, the BTCs of prosulfocarb in S columns were asymmetrical and different to those of the chloride ion. They recorded a rapid initial leaching of ^{14}C applied to the column for a PV ≤ 1.50, although the BTCs recorded a long tail with shoulders (saturated flow) (**Fig. 1A**) or peaks (saturated-unsaturated flow) (**Fig. 1B**), indicating the steady leaching of the herbicide when up to 500 mL (11PV) of water were pumped under both flow conditions. These asymmetrical BTCs were also reported for other herbicides and fungicides in an unamended soil (Khorram et al., 2017; Marín-Benito et al., 2018c). The peak concentration was recorded at a water volume between 1.10 and 1.28 PV, similar to that recorded for the tracer ion, albeit lower (**Table 2**; **Fig. 1A,B**). This peak represents 2.01%-6.33% of the total ^{14}C applied to the S columns for the non-incubated herbicide under saturated or saturated-unsaturated flow, respectively.

When the herbicide was incubated in the soil column for 28 days, the BTC pattern was similar, albeit with lower peak concentrations. Concentrations decreased up to ~2-3 times compared to those of the non-incubated herbicide (**Table 2; Fig. 1A,B**). Changes in peak concentrations with incubation time were also observed for the leaching of other pesticides in an unamended soil (Álvarez-Martín et al., 2017), although the decrease was not always as significant as observed here.

In the S+GC columns, BTCs recorded early peaks when a saturated flow was applied; the mobility of prosulfocarb was then very slow, and the leaching of ^{14}C was continuously determined up to a water volume of 9-11 PV (**Table 2**). However, the BTCs for prosulfocarb leaching in S+GC columns under saturated-unsaturated flow were different because they recorded a long tail with several peaks. The early peaks of BTCs were recorded for a water volume between 0.79 and 1.50 PV under both flow conditions, similar to those found for the herbicide in the unamended soil and for the chloride ion. The leaching kinetic of prosulfocarb was faster, however, and peak concentration was higher in S+GC (2.99%) than in S under saturated flow, although the adsorption of prosulfocarb by S+GC was higher than by S (**Table 1**). A similar effect was observed for the leaching of the hydrophobic compound tebuconazole in an amended soil using similar experimental conditions (Álvarez-Martín et al., 2017). The DOC content in S+GC (0.027%) was higher than in S (0.006%), and it favoured the compound's initial rapid movement in the column. This effect of DOM or DOC on the adsorption of hydrophobic compounds increasing their mobility has been reported in previous works (García-Jaramillo et al., 2014; Marín-Benito et al., 2018b; Thevenot et al., 2009). There is a decreasing or increasing effect of DOC on the adsorption of hydrophobic compounds by the batch equilibrium technique and on the mobility of compounds under saturated water conditions (Marín-Benito et al., 2016). The effect of rapid leaching was also observed in the early peak concentration for the non-incubated prosulfocarb under saturated-unsaturated flow conditions (4.34%) (**Fig. 1C,D**), although a second peak with a concentration of 2.72% (4.98 PV) was recorded under this saturated-unsaturated flow, and the leaching pattern was closer to that recorded in the unamended soil. This behaviour could be attributed to non-equilibrium sorption due to the time-dependent interactions that occur between the herbicide and soil components and/or organic amendment, which may be more difficult to attain under the conditions of unsaturated water flow (Brusseau et al., 1992; Dousset et al., 2007; Marín-Benito et al., 2009). This asymmetrical behaviour is also commonly observed for other pesticides in amended soils (Fenoll et al., 2014; Khorram et al., 2015; Rodríguez-Cruz et al., 2011).

Peak concentrations decreased after herbicide incubation in the S+GC columns under saturated and saturated-unsaturated flow regimes. They fell within the range 0.65%-1.31% of the total ^{14}C applied to the column for the incubated compound. The first peak of prosulfocarb after incubation is broad and not well defined under saturated-unsaturated flow (**Fig. 1 D**) (**Table 2**). A second peak with concentrations of 1.59% (3.34 PV) was also recorded when incubated herbicide was leached under saturated-unsaturated flow. The BTCs patterns were similar for prosulfocarb leaching when no incubation was carried out and after the incubation of the herbicide in the S+GC column (**Fig. 1C,D**). However, the peak concentrations of herbicide were ~4.6 times and ~3.3 times lower after incubation under saturated or saturated-unsaturated flow, respectively. This decrease in herbicide concentration under all conditions could be due to the increase in the mineralized amount or to the possible formation of bound residues of ^{14}C-herbicide after incubation in soil columns compared to non-incubated ones (**Table 2**), consistent with the dissipation mechanisms of prosulfocarb in S and S+GC described by Barba et al. (2019).

3.3. Leaching of prosulfocarb in unamended and amended soil columns

The total amounts leached for non-incubated prosulfocarb after the application of 10-11 PV to the S columns were 13.0% and 26.7% for saturated and saturated-unsaturated flow, respectively. For the S+GC columns, these values were similar in the case of saturated flow (12.2%) and slightly higher than the one obtained in saturated-unsaturated flow (32.5%). A higher movement of prosulfocarb through the soil profile in the GC-amended soil than in the unamended soil was also recorded in a field experiment (Marín-Benito et al., 2018b).

The leached amounts decreased when the herbicide was incubated for 28 days in the S and S+GC columns. They decreased 2.1 and 1.9 times for the S columns, and 2.9 and 1.6 times for the S+GC columns under saturated or saturated-unsaturated flow, respectively (**Table 2**). Walker et al. (2005) indicated that time-dependent sorption processes are important for controlling pesticide movement in soil. The time elapsed between the application and leaching of the herbicide could explain these results, as the losses of herbicides following their application were higher than when leaching events occur days after an incubation period (Milan et al., 2015). Results obtained are consistent with the prosulfocarb dissipation in the same S and S+GC soils reported by Barba et al. (2019). They found that after a 27-day incubation period the fraction extracted with a 0.01M $CaCl_2$ water solution (potentially leachable) decreased by up to 7% in the unamended soil, being higher in S+GC than in S. Previous studies have also reported a decrease in tebuconazole and cymoxanil leaching after pesticide incubation in an experiment with packed soil columns (Álvarez-Martín et al., 2017).

3.4. Retention of prosulfocarb in unamended and amended soil columns

The total amounts retained after herbicide leaching were lower in S than in S+GC under saturated flow. However, these amounts were similar in both S and S+GC when saturated-unsaturated flow was applied. These values were the same after incubating the herbicide in the column (**Table 2**). They are consistent with the higher adsorption constant of prosulfocarb by S+GC than by S, as previously indicated (**Table 1**), suggesting a stronger interaction between the herbicide and the S+GC soil. Previous leaching studies have reported a higher retention of pesticides by amended soils than by unamended ones (Ghosh and Singh, 2012; Jiang et al., 2016; Khorram et al., 2015; Larsbo et al., 2013). Prosulfocarb retention was higher under saturated than saturated-unsaturated flow for the non-incubated and incubated compounds in agreement with the corresponding amounts leached (**Table 2**), with this effect contradicting our results reported for other pesticides (Álvarez-Martín et al., 2017). The slow leaching kinetic of prosulfocarb in S and S+GC under saturated-unsaturated flow could increase herbicide degradation. The initial amounts retained could not remain in both soils if prosulfocarb metabolites were produced over time with a high leachability capacity. Prosulfocarb has a relatively short half-life, and the formation of degradation products has been reported (Barba et al., 2019;

Braun et al., 2017). Degradation could be enhanced by the organic matter in S+GC, according to the higher amount leached in this soil than in the unamended one (**Table 2**).

The distribution of the ^{14}C amounts retained in the different segments of the columns, expressed as a percentage of the total ^{14}C applied to the columns under all the conditions (**Fig. 2**), indicated that prosulfocarb was primarily retained in the first segment of the columns. The retention in the S columns was > 28% in all cases, and in the S+GC columns it also occurred mainly in the first segment, > 74% under saturated flow, and ~43% under saturated-unsaturated flow, according to the total amount retained as previously indicated. A higher percentage of pesticides remaining in the first segment of the column for unamended soils and soils amended with biochar or fly ash has usually been reported (Gámiz et al., 2017; Ghosh and Singh, 2012; Khorram et al., 2015). The amounts of herbicide present in the lower segments of the S columns were higher than in the S+GC columns. This suggests a stronger binding of the herbicide with the organic amendment in the upper layer, or the formation of bound residues with the soil (Barriuso et al., 2008), which would cause the retention in the upper layer of the column (García-Jaramillo et al., 2014; Marín-Benito et al., 2009). Prosulfocarb may be able to form bound residues, as reported in our previous work (Barba et al., 2019), but these residues may be bioavailable for degradation under saturated-unsaturated flow, as previously indicated, decreasing the remaining prosulfocarb in the first segment and increasing the total amount leached.

3.5. Mineralization of Prosulfocarb in unamended and amended soil columns

The total mineralized amounts of non-incubated ^{14}C-prosulfocard were low (≤ 0.70%) in the S and S+GC columns under saturated flow (**Table 2**). However, under the saturated-unsaturated flow condition, the mineralized amounts were higher (11.3%-

14.9%), probably due to the longer leaching experiment (20 days). In a previous dissipation work, the mineralization of prosulfocarb was > 18% after 27 days incubation in S and > 14% in S+GC (Barba et al., 2019). As regards the incubated herbicide in the soil columns, the total mineralized amounts increased up to 19 times under saturated flow compared to the non-incubated herbicide in the soil column. These results suggest that incubation time did not greatly affect the herbicide's retention, but it significantly increased the mineralized amount under saturated flow. As regards the incubated herbicide in the S and S+GC columns, the mineralized amounts were similar under saturated-unsaturated flow compared to the non-incubated herbicide (**Table 2**).

3.6. Total balance and retardation factors of prosulfocarb in unamended and amended soil columns

The total amounts of prosulfocarb leached, retained and mineralized were determined to evaluate the total balance of ^{14}C-prosulfocarb in the columns. They were expressed as the percentages of the total ^{14}C initially added to the columns, and the values found were between 73% and 88% in the S columns, and between 80% and 97% in the S+GC columns (**Table 2**). Prosulfocarb is considered a slightly volatile compound, so some losses due to volatilization are expected (EFSA, 2007). Prosulfocarb volatilization from soil has been reported (Carlsen et al., 2006), and it has been detected in rainwater or the air (Khalil et al., 2019; Kreuger et al., 2017). The possible volatilization of prosulfocarb after incubation occurred in the S column, and to a lesser extension in the S+GC column. These results are consistent with our previous work, where we studied the dissipation mechanism of prosulfocarb, finding that the total ^{14}C mass balance was 92.4% and 94.9% at time 0 days for S and S+GC soils, respectively, with these percentages decreasing over time (Barba et al., 2019).

Finally, the retardation factors (R) were determined for comparing the BTCs under different conditions (**Table 1**). Their values ranged from 26.3 to 28.2 in S, and from 30.6 to 32.7 in S+GC. The R factors for S+GC are slightly higher than for S soils, but this is not reflected in the PV values corresponding to the initial peaks due to the BTC patterns with various peaks. The R results indicate a lower mobility of prosulfocarb in the S+GC column, although this was not the case for the saturated-unsaturated flow. The high R values found for unamended and amended soils indicate the low leaching potential of prosulfocarb and the highest percentage of herbicide retained under all the conditions assayed.

4. Conclusions

The results obtained in this work show the effects that the application of GC to soil had in the leaching of the herbicide prosulfocarb when different water flow and herbicide ageing conditions were applied. Although the total amount of prosulfocarb leached differed little between unamended and amended soils, the amount of herbicide retained was higher in the amended soil under saturated flow (1.4 times). The amendment had less impact when the saturated-unsaturated water flow was applied after the non-incubation or incubation of the herbicide. However, the presence of the organic amendment decreased the loss of prosulfocarb due to other processes such as volatilization. When the leaching experiment was carried out after 28 days of incubation, the amount of prosulfocarb leached decreased for both the unamended and amended soils, although the amount mineralized increased. The results on the leaching of prosulfocarb from this study complement the field studies carried out in previous works. Several factors, such as amendment, water flow and herbicide ageing, which are related to climate and agronomic factors, have proven to be important for controlling the leaching of this herbicide through the soil profile.

Declarations of interest: none.

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Figure Captions

Fig. 1. Breakthrough curves for the leaching of conservative chloride ion (Cl^-) and prosulfocarb (% of the initially ^{14}C-herbicide applied) without incubation (NI) and after incubation (I) in unamended soil columns (S) (A,B) and in soil columns amended with green compost (S+GC) (C,D) under different flow regimes.

Fig. 2. Amounts and distribution of prosulfocarb without incubation (NI) and after incubation (I) retained in unamended soil columns (S) and in soil columns amended with green compost (S+GC) under different flow regimes.

Table 1. Adsorption coefficients (K), pore volume (PV), density (ρ), porosity (θ) and retardation factor (R) for prosulfocarb in unamended (S) or amended soils (S + GC).

Column / Flow	Incubation	K[a] ± SD[b] (mL g^{-1})	PV ± SD (mL)	ρ ± SD (g cm^{-3})	θ ± SD (cm^3 cm^{-3})	R ± SD
S		12.3 ± 0.10				
Sat.[c]	Non-incubated		45.1 ± 2.10	1.17 ± 0.01	0.53 ± 0.03	28.2 ± 1.27
Sat-Unsat.[d]	Non-incubated		48.4 ± 0.69	1.18 ± 0.00	0.57 ± 0.01	26.3 ± 0.36
Sat.	Incubated		48.4 ± 6.45	1.18 ± 0.01	0.57 ± 0.07	26.6 ± 3.41
Sat-Unsat.	Incubated		46.2 ± 0.63	1.16 ± 0.03	0.54 ± 0.02	27.5 ± 0.36
S+GC		16.9 ± 0.27				
Sat.	Non-incubated		56.3 ± 4.65	0.91 ± 0.03	0.55 ± 0.06	31.1 ± 2.49
Sat-Unsat.	Non-incubated		55.5 ± 3.99	1.03 ± 0.03	0.57 ± 0.06	31.5 ± 2.19
Sat.	Incubated		57.1 ± 0.36	1.03 ± 0.00	0.59 ± 0.00	30.6 ± 0.19
Sat-Unsat.	Incubated		53.4 ± 0.98	1.08 ± 0.14	0.58 ± 0.06	32.7 ± 0.58

[a] Adsorption coefficients determined from linearized isotherms (unpublished data).

[b] Standard deviation of replicates (n = 3).

[c] Saturated.

[d] Saturated-unsaturated.

Table 2. Total amounts of retained, leached and mineralized prosulfocarb (% of ^{14}C applied) in unamended (S) and amended (S+GC) soil columns at different flow conditions and incubation time of herbicide before leaching.

Parameters	Non-incubated herbicide		Incubated herbicide	
	Saturated	Saturated-unsaturated	Saturated	Saturated-Unsaturated
S				
Max. peak	2.01 ± 1.20[a]	6.33 ± 0.43	0.87 ± 0.64	1.85 ± 0.05
		2.63 ± 0.14		
PV	1.10 ± 0.20	1.25 ± 0.02	1.27 ± 0.15	1.28 ± 0.09
		2.46 ± 0.03		
Total retained[b]	60.8 ± 11.4a	50.5 ± 1.15a	57.1 ± 1.57a	43.3 ± 2.80a
Total leached	13.0 ± 2.67b	26.7 ± 1.57c	6.17 ± 0.24a	14.2 ± 1.13b
Total mineralized	0.70 ± 0.20a	11.3 ± 1.10b	12.0 ± 1.18b	15.8 ± 1.87b
Total column	74.5 ± 13.9a	88.5 ± 1.97a	75.3 ± 0.15a	73.3 ± 0.19a
S+GC				
Max. peak	2.99 ± 0.25	4.34 ± 0.96	0.65 ± 0.13	1.31 ± 0.35
		2.72 ± 0.04		1.59 ± 0.29
PV	1.34 ± 0.01	1.47 ± 0.10	1.41 ± 0.12	1.50 ± 0.01
		4.98 ± 0.34		3.34 ± 0.03
Total retained[b]	85.2 ± 7.23b	47.6 ± 2.43a	77.4 ± 5.88b	47.7 ± 7.07a
Total leached	12.2 ± 4.56ab	32.5 ± 1.54c	4.21 ± 1.20a	20.0 ± 4.75bc
Total mineralized	0.40 ± 0.03a	14. 9 ± 2.31c	7.60 ± 1.20b	12.8 ± 1.36bc
Total column	97.8 ± 3.02b	95.0 ± 3.20b	89.3 ± 5.19ab	80.5 ± 0.10a

[a] Standard deviation of replicates (n = 3).

[b] Different letters in the same line indicate significant differences in retained, leached, mineralized and total amounts among soil columns with different herbicide incubation and irrigation regimes ($p < 0.05$).

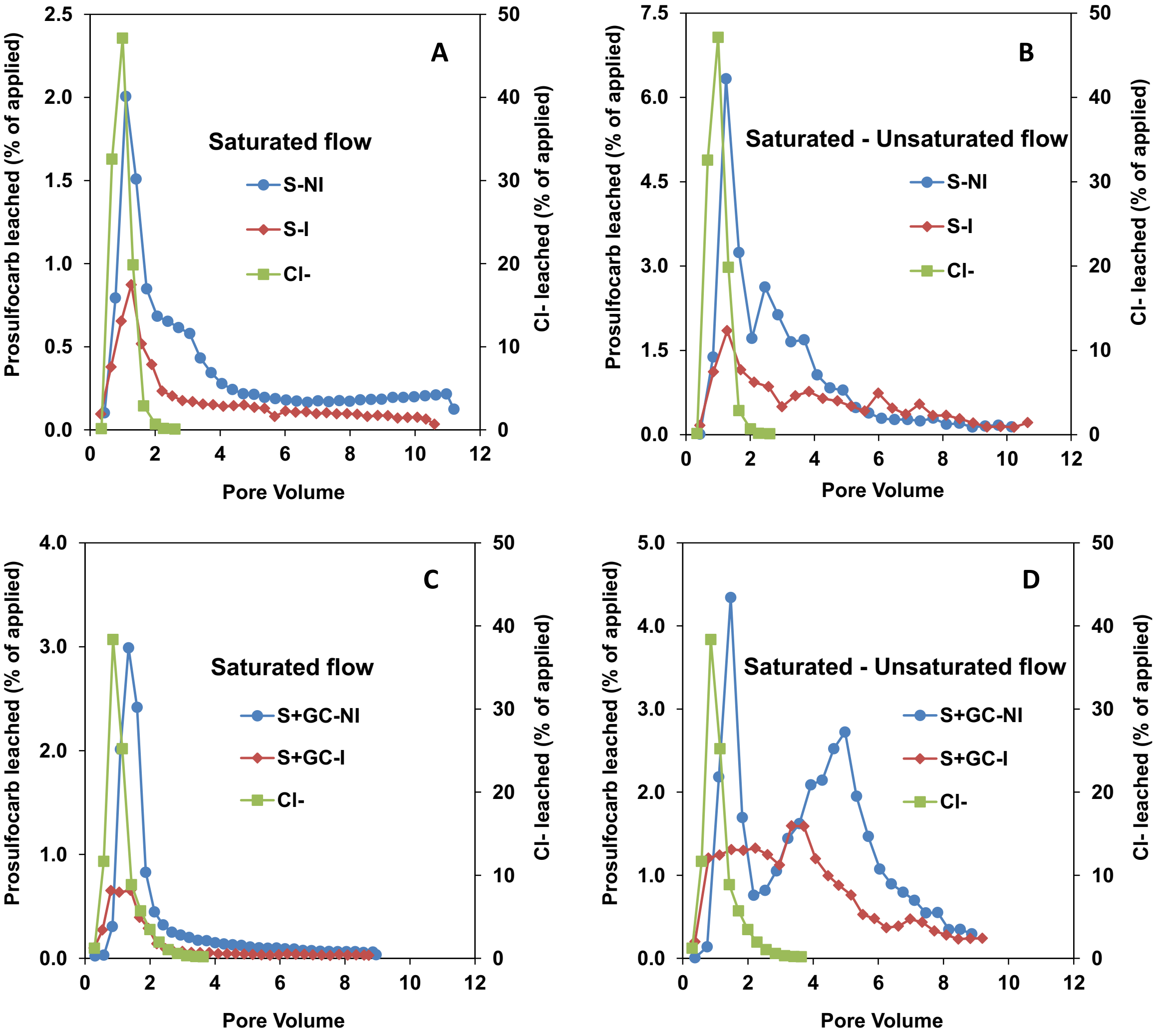
A
Saturated flow
S-NI
S-I
Cl-
Prosulfocarb leached (% of applied)
Cl- leached (% of applied)
Pore Volume
0.0
0.5
1.0
1.5
2.0
2.5
0
10
20
30
40
50
0
2
4
6
8
10
12
B
Saturated - Unsaturated flow
S-NI
S-I
Cl-
Prosulfocarb leached (% of applied)
Cl- leached (% of applied)
Pore Volume
0.0
1.5
3.0
4.5
6.0
7.5
C
Saturated flow
S+GC-NI
S+GC-I
Cl-
Prosulfocarb leached (% of applied)
Cl- leached (% of applied)
Pore Volume
0.0
1.0
2.0
3.0
4.0
D
Saturated - Unsaturated flow
S+GC-NI
S+GC-I
Cl-
Prosulfocarb leached (% of applied)
Cl- leached (% of applied)
Pore Volume
0.0
1.0
2.0
3.0
4.0
5.0

Figure 1

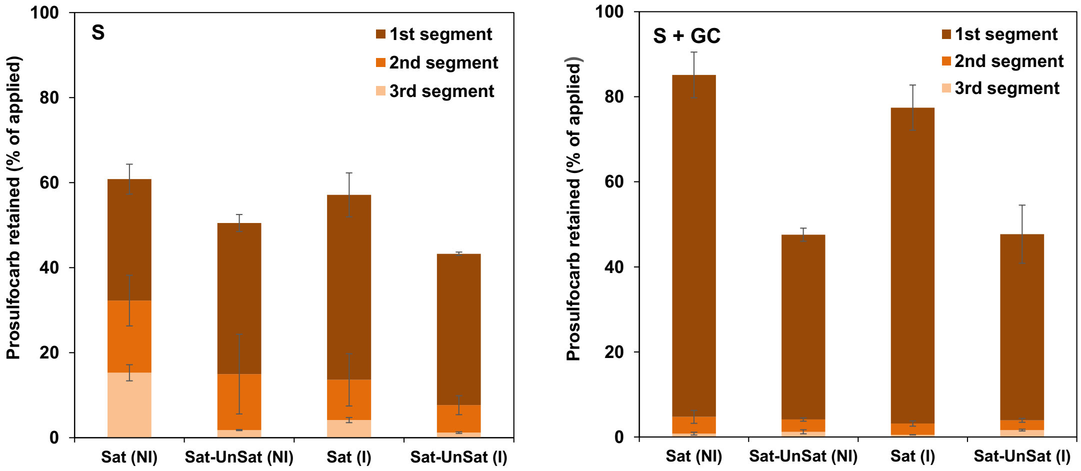
S
S + GC
1st segment
2nd segment
3rd segment
Prosulfocarb retained (% of applied)
0
20
40
60
80
100
Sat (NI)
Sat-UnSat (NI)
Sat (I)
Sat-UnSat (I)

Figure 2

Material Suplementario

SUPPLEMENTARY MATERIAL

Transport of ^{14}C-prosulfocarb through soil columns under different amendment, herbicide incubation and irrigation regimes

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Table S1. Characteristics of unamended soil, green compost and green compost-amended soil.

Samples	pH	OC[a] (%)	DOC[b] (%)	N (%)	C/N
Soil	7.35	1.30	0.006	0.12	10.8
Green compost	7.20	24.1	0.703	1.11	21.8
Soil+Green compost	7.30	4.66	0.027	0.42	11.1

[a] Organic carbon.

[b] Dissolved organic carbon.

Table S2. Factor studied in the leaching of prosulfocarb in packed soil columns. Four herbicide leaching experiments were performed in triplicate for each soil treatment (unamended and green compost-amended soils).

Soil treatment	**Incubation**	**Flow regime**
Unamended Soil (S)	Non-incubated columns (NI) (leaching 1 day after herbicide application)	Saturated (500 mL at 1 mL min^{-1})
		Saturated – Unsaturated (500 mL at 20 mL day^{-1})
	Incubated columns (I) (leaching 28 days after herbicide application)	Saturated (500 mL at 1 mL min^{-1})
		Saturated – Unsaturated (500 mL at 20 mL day^{-1})
Green Compost amended Soil (S+GC)	Non-incubated columns (NI) (leaching 1 day after herbicide application)	Saturated (500 mL at 1 mL min^{-1})
		Saturated – Unsaturated (500 mL at 20 mL day^{-1})
	Incubated columns (I) (leaching 28 days after herbicide application)	Saturated (500 mL at 1 mL min^{-1})
		Saturated – Unsaturated (500 mL at 20 mL day^{-1})

CONCLUSIONES

CONCLUSIONES

Estudios bajo condiciones de campo

1. La aplicación de compost vegetal disminuyó la disipación del herbicida triasulfuron en el suelo enmendado respecto al suelo sin enmendar cuando se aplicó como formulación individual, aunque este efecto no se observó en la formulación conjunta. La disipación de prosulfocarb fue más rápida que la de triasulfuron, y no se encontraron diferencias significativas con las diferentes formulaciones aplicadas. La disipación de prosulfocarb, más hidrofóbico que triasulfuron, estuvo posiblemente controlada por una adsorción irreversible por el suelo independientemente del tratamiento.

2. El estudio de movilidad de triasulfuron a través del perfil del suelo inicialmente indicó la retención del herbicida en el tramo superficial del suelo, aunque también se determinaron concentraciones relevantes en el tramo 10-20 cm en todas las condiciones ensayadas. En presencia del compost vegetal, se detectó un descenso de la lixiviación de triasulfuron cuando se aplicó como formulación individual, pero no cuando se hizo bajo formulación combinada con prosulfocarb movilizándose en ese caso hasta una profundidad de 50 cm. Se constató una estrecha relación entre el contenido de agua en el perfil del suelo y la lixiviación de triasulfuron.

3. Para prosulfocarb, la mayor retención también se detectó siempre en el tramo superficial mientras que pequeñas concentraciones del herbicida se determinaron en el tramo 10-20 cm en el suelo enmendado con compost vegetal. No se observó el efecto de la formulación individual o combinada en la movilidad de prosulfocarb. Tampoco se constató influencia del contenido de agua en el perfil del suelo en la lixiviación. La movilidad de este herbicida hidrofóbico detectada hasta 50 cm en el perfil del suelo podría estar influenciada por el carbono orgánico disuelto derivado del compost vegetal.

4. La aplicación de los herbicidas triasulfuron y prosulfocarb modificó la actividad y estructura microbiana del suelo, especialmente en los suelos sin enmendar. Individualmente, triasulfuron registró un mayor impacto en la biomasa microbiana y respiración del suelo que prosulfocarb. La aplicación combinada de ambos herbicidas en el suelo sin enmendar produjo un cambio en su estructura microbiana mientras que el compost vegetal amortiguó el impacto de los herbicidas aplicados conjuntamente en la biomasa y actividad, y redujo los cambios en la estructura microbiana. Sin embargo, a

pesar del efecto amortiguador del compost, la aplicación combinada de los herbicidas tuvo mayor impacto en la biomasa y actividad microbiana del suelo que la aplicación individual de los mismos.

5. El aumento de la dosis de compost vegetal aplicado en el suelo dio lugar a una disipación más lenta de los herbicidas y a un aumento de la persistencia de ambos compuestos. Este comportamiento fue consecuencia de la mayor adsorción de estos compuestos por los suelos debido a su mayor contenido en carbono orgánico.

6. La aplicación de riego adicional incrementó la movilidad de ambos herbicidas, viéndose además potenciada por el incremento del carbono orgánico disuelto, especialmente para prosulfocarb. Sin embargo, la mayor capacidad de retención de agua del suelo enmendado con la dosis mayor de compost vegetal podría reducir la movilidad de los herbicidas.

7. La aplicación repetida de los herbicidas produjo un aumento de su velocidad de disipación especialmente para el triasulfuron, aunque en el caso del prosulfocarb las cantidades residuales persistieron durante más tiempo tras la segunda aplicación. Una degradación acelerada de los herbicidas por parte de los microorganismos ya adaptados podría ocurrir, permitiendo un metabolismo más rápido de los mismos. Además, el incremento de temperatura registrado (~10ºC) durante la segunda aplicación podría haber acelerado el proceso de disipación de los herbicidas y/o una mayor volatilización en el caso del prosulfocarb.

8. La aplicación repetida de los herbicidas produjo cambios en la estructura microbiana de los suelos sin enmendar y enmendados. Se encontró una correlación positiva entre las cantidades de residuos de herbicidas y la población microbiana total, indicando una disminución de esta población durante la disipación de herbicidas y una cierta toxicidad de los herbicidas para la comunidad microbiana. Los herbicidas aumentaron la población relativa de *Actinobacterias* y redujeron la población relativa de hongos en comparación con la situación inicial en todas las condiciones estudiadas. La población de *Actinobacterias* parece ser responsable del incremento de la degradación de triasulfuron después de la segunda aplicación.

Estudios bajo condiciones de laboratorio

9. Los estudios de disipación, movilidad y efecto en las comunidades microbianas del prosulfocarb llevados a cabo en condiciones de laboratorio apoyaron los resultados obtenidos en condiciones de campo. La disipación y biodisponibilidad del herbicida fueron, al igual que en el experimento de campo, más elevadas en el suelo sin enmendar que en el suelo enmendado, independientemente de la concentración aplicada. Sin embargo, se observó una *fase lag* en la disipación de prosulfocarb aplicado a dos dosis en el suelo enmendado con compost vegetal que retrasó su biodegradación.

10. El estudio del mecanismo de disipación de ^{14}C-prosulfocarb indicó una disminución de las cantidades extraíbles del compuesto con el tiempo indicando un envejecimiento del herbicida en el suelo. Por otra parte, las fracciones mineralizadas y no extraíbles aumentaron lentamente con el tiempo de incubación. El balance de masa total de ^{14}C-prosulfocarb indicó la pérdida de parte del herbicida por volatilización u otros procesos no controlados en el experimento.

11. La presencia del prosulfocarb no mostró efectos negativos en la actividad deshidrogenasa, lo que indica que no presenta un impacto negativo significativo sobre la actividad microbiana. Por el contrario, el herbicida estimuló esta actividad en el suelo enmendado con el tiempo debido a la mayor cantidad de herbicida extraíble en comparación con el suelo sin enmendar. Además, éste tuvo un impacto limitado sobre la estructura microbiana, pero sí estuvo claramente influenciada por la aplicación del compost vegetal.

12. La movilidad del herbicida prosulfocarb en columnas de suelo empaquetadas indicó que el compost vegetal sólo modificó la lixiviación de prosulfocarb en el suelo bajo flujo saturado-no saturado. La enmienda orgánica sí tuvo efecto para aumentar la cantidad de herbicida retenido bajo los dos regímenes de flujo estudiados y disminuyó además su pérdida vía otros procesos como la volatilización. La cantidad de herbicida lixiviado bajo los dos tipos de flujo disminuyó en los suelos sin enmendar y enmendados cuando el compuesto fue previamente incubado. Sin embargo, la cantidad de herbicida mineralizado aumentó para el flujo saturado-no saturado, pero fue similar en el caso de flujo saturado.

13. Prosulfocarb sería un herbicida recomendable para el control de malas hierbas debido a su rápida degradación y a su baja toxicidad para la microbiología del suelo. Factores como la enmienda, el flujo de agua y el envejecimiento del herbicida, que están íntimamente relacionados con el clima y factores agronómicos, han resultado ser importantes en el control de la lixiviación de este herbicida a través del perfil del suelo.

En resumen, a partir de los resultados obtenidos se podría concluir que:

i) La movilidad de los herbicidas por debajo del horizonte superficial del suelo podría acortar la duración del control residual de las malas hierbas a las que van destinados y dar lugar a contaminaciones de las aguas subterráneas. Por otro lado, las cantidades retenidas en el perfil del suelo podrían exceder el umbral de sensibilidad registrado para cultivos susceptibles con el consiguiente daño en cosechas posteriores.

ii) Es necesario llevar a cabo estudios por un período de tiempo más largo que permitan establecer la influencia de las nuevas prácticas agrícolas basadas en la aplicación de residuos orgánicos al suelo sobre el comportamiento de los herbicidas y sobre la estructura microbiana.

iii) Es necesario evaluar las características de los residuos orgánicos previamente a su aplicación, especialmente su contenido en carbono orgánico total y soluble, así como los cambios estructurales en los agregados del suelo con el fin de mejorar su fertilidad sin aumentar el impacto ambiental cuando su aplicación coincida con la de los herbicidas.

iv) La aplicación de compost vegetal como enmienda orgánica podría ser optimizada previamente a su uso para reducir el riesgo de contaminación de aguas subterráneas por lixiviación de herbicidas y minimizar el impacto de los herbicidas sobre la microbiota del suelo.