Ecotoxicology and Environmental Safety 182 (2019) 109395

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

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[1], María J. Sánchez-Martín, M. Sonia Rodríguez-Cruz*

Institute of Natural Resources and Agrobiology of Salamanca (IRNASA-CSIC), Cordel de Merinas 40-52, 37008, Salamanca, Spain

ARTICLE INFO

Keywords:
Prosulfocarb
Soil
Green compost
Dissipation
Mass balance
Microbial community

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

* Corresponding author.
E-mail address: msonia.rodriguez@irnasa.csic.es (M.S. Rodríguez-Cruz).
[1] Present address: Department of Geology and Geochemistry, Autonomous University of Madrid, 28049, Madrid, Spain

https://doi.org/10.1016/j.ecoenv.2019.109395
Received 22 March 2019; Received in revised form 24 June 2019; Accepted 26 June 2019
Available online 01 July 2019

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

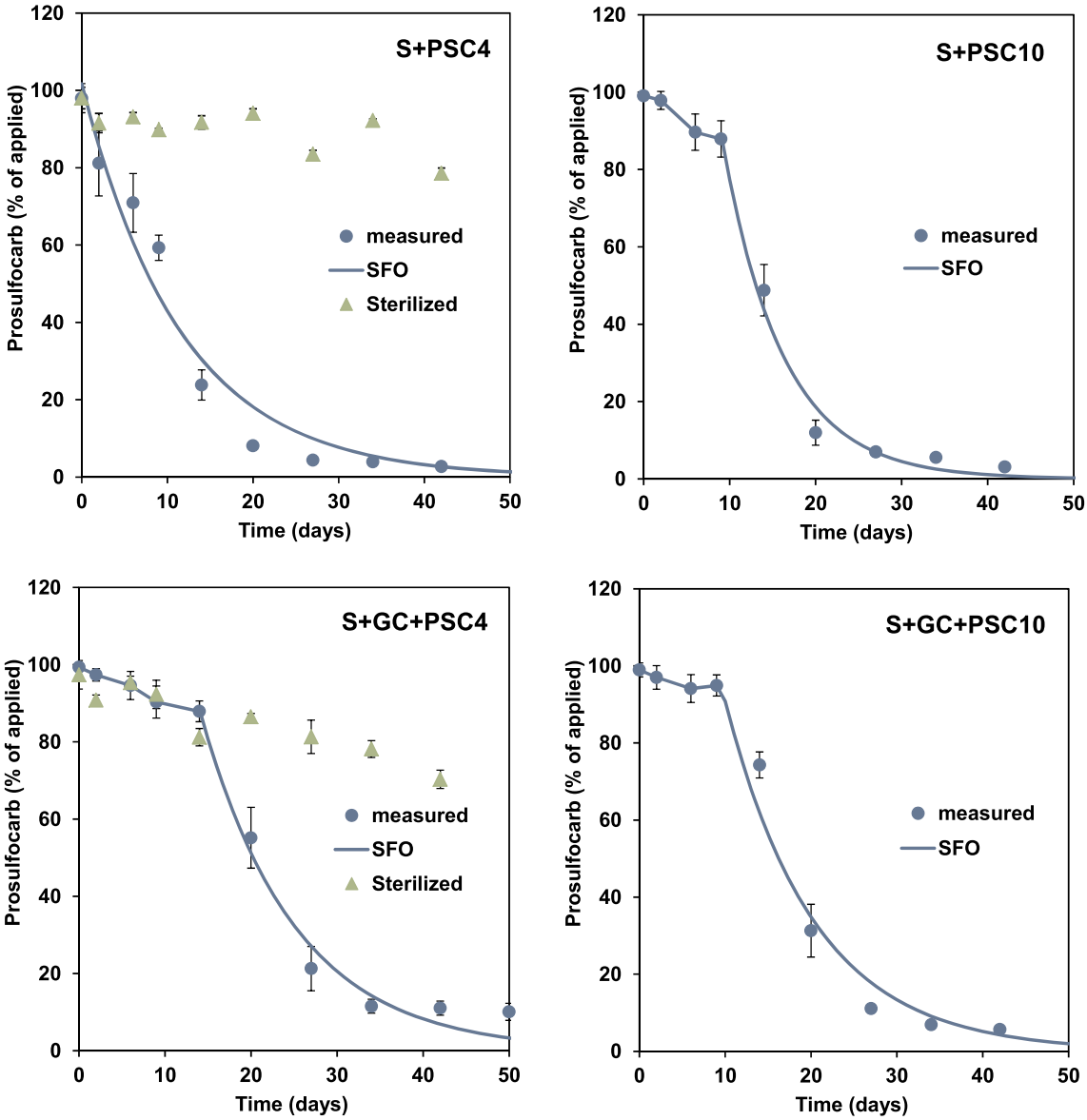

Fig. 1. Dissipation kinetics of non-labeled prosulfocarb (PSC) applied at two doses in unamended (S + PSC4 and S + PSC10) and GC-amended soils (S + GC + PSC4 and S + GC + PSC10). Error bars indicate the standard deviation of the mean value (n = 4).

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.

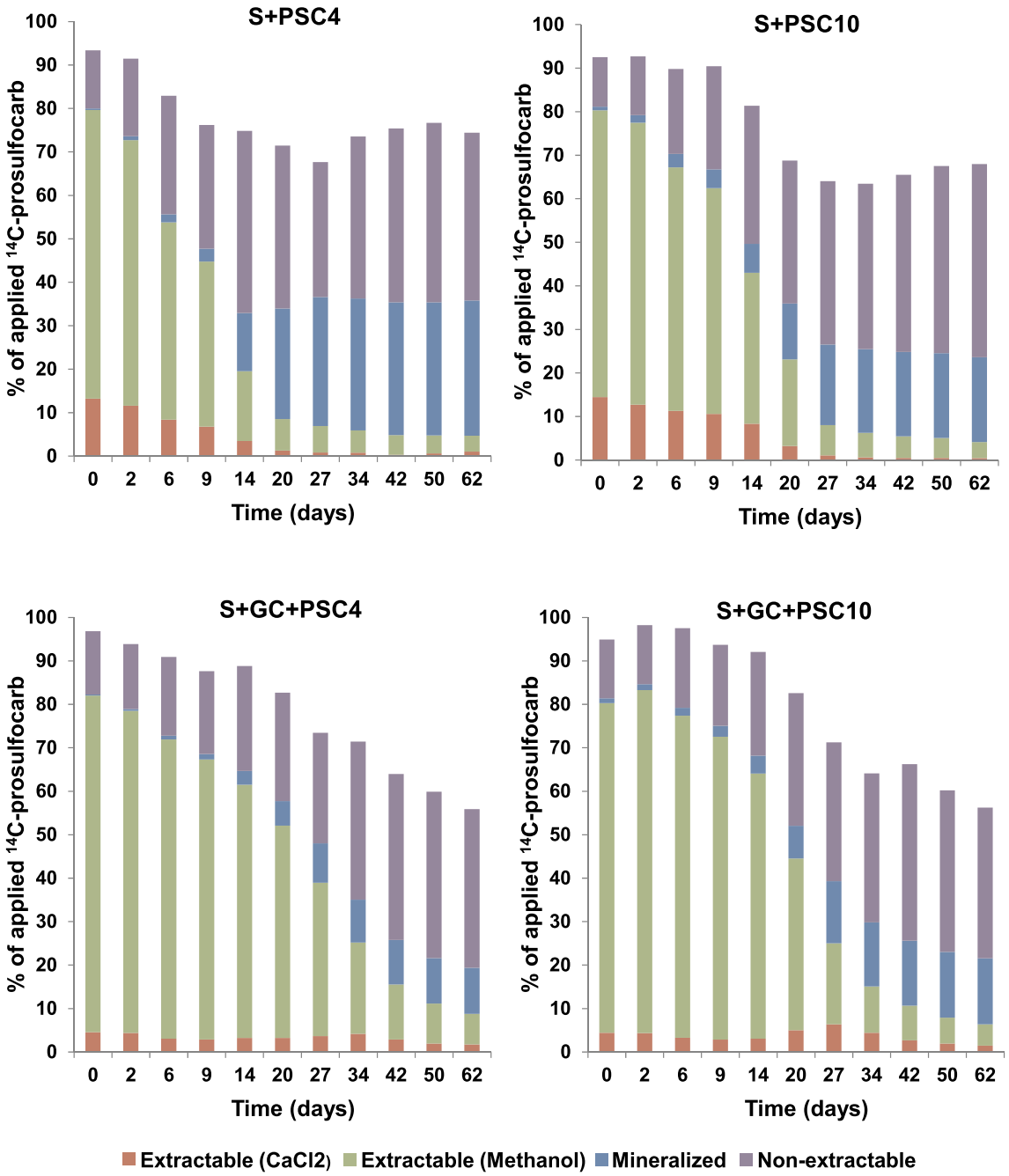

Fig. 2. $CaCl_2$-extractable, MeOH-extractable, mineralized and non-extractable fractions of ^{14}C-prosulfocarb applied at two doses in unamended (S + PSC4 and S + PSC10) and GC-amended soils (S + GC + PSC4 and S + GC + PSC10).

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-

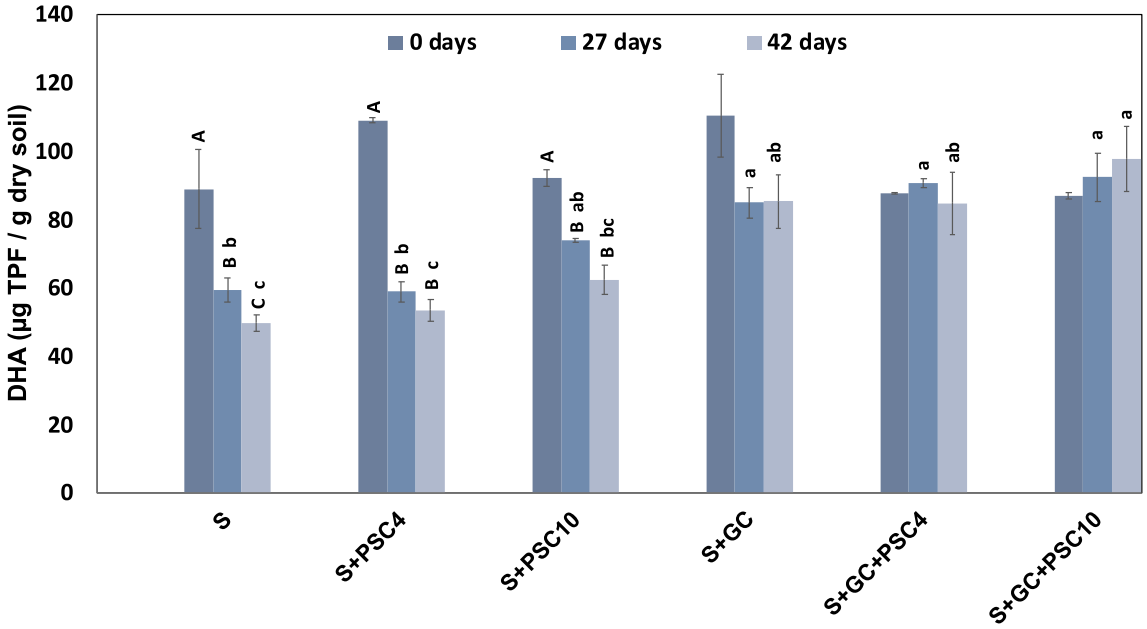

Fig. 3. Soil dehydrogenase activity (DHA) for unamended (S) and GC-amended soil (S + GC) untreated and treated with prosulfocarb at two doses (PSC4 and PSC10) at 0, 27 and 42 days. Bars indicate the standard deviation of the mean (n = 4). Different lowercase and uppercase letters indicate significant 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

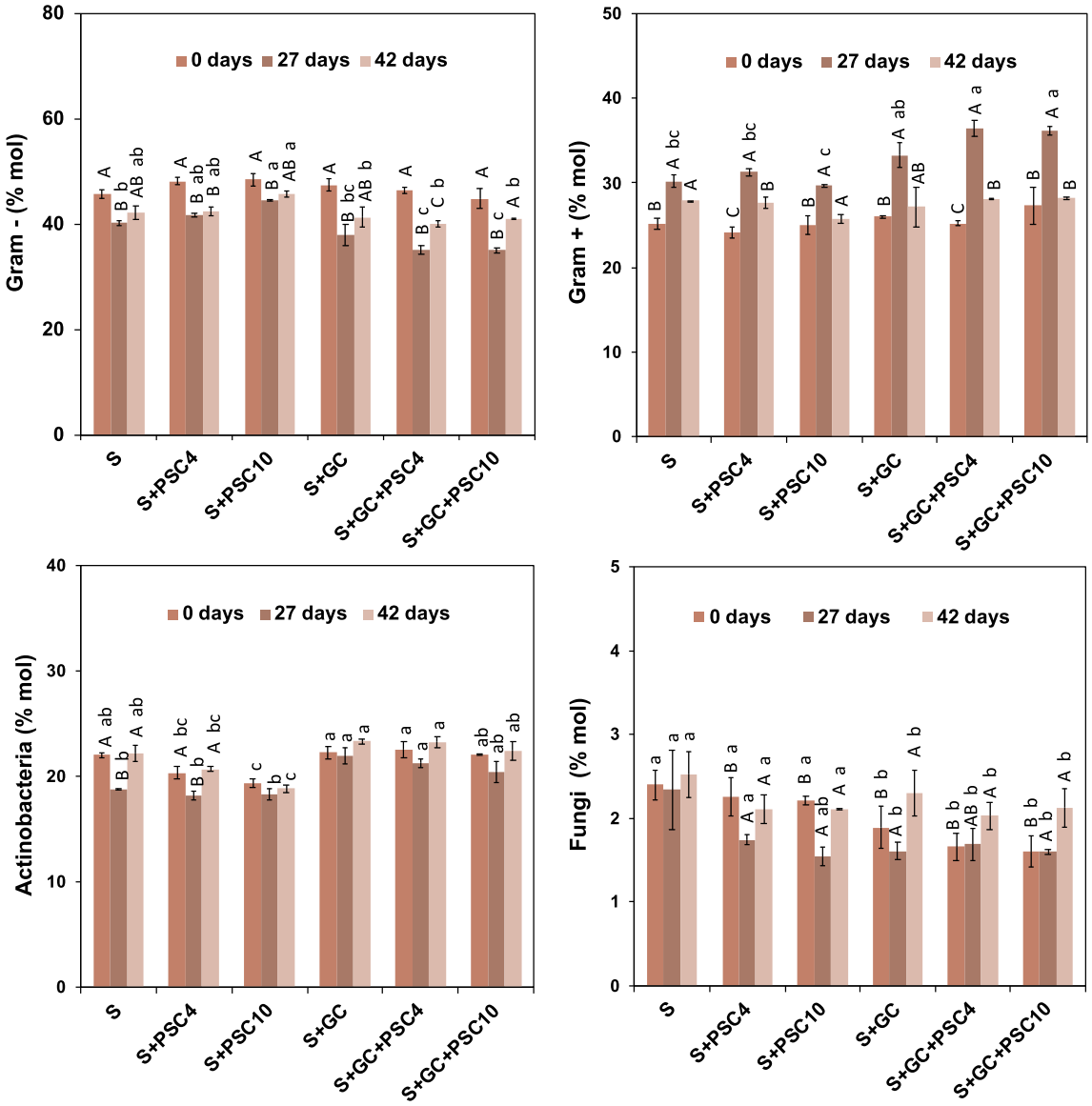

Fig. 4. Relative abundance (% mol) of PLFAs specifically diagnostic of Gram-negative and Gram-positive bacteria, *Actinobacteria* and fungi in unamended (S) and GC-amended soil (S + GC) untreated and treated with prosulfocarb at two doses (PSC4 and PSC10) at 0, 27 and 42 days. Bars indicate the standard 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

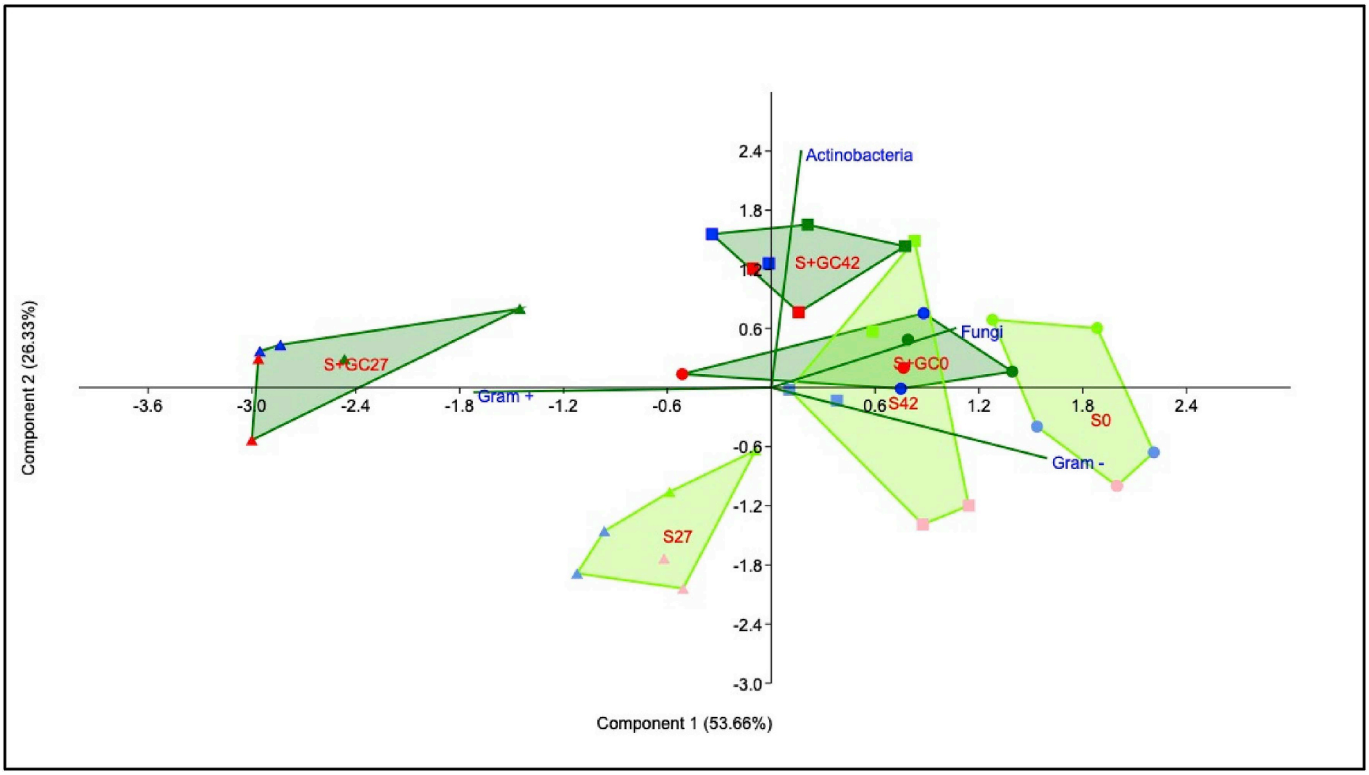

Fig. 5. Principal component analysis (PCA) showing loading scores for Gram-negative and Gram-positive bacteria, *Actinobacteria*, fungi, soil DHA, soil humidity, and percentage of remaining prosulfocarb and sampling time and scores of each treatment (control: green; low dose: blue; high dose: red) and 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.

Acknowledgments

This work was funded by the Regional Government Junta de Castilla y León (project CSI240U14) and by the Provincial Government of Salamanca. V. Barba thanks “Junta de Castilla y León” for his predoctoral contract. J.M. Marín-Benito and C. García-Delgado thank Ministry of Economy and Competitiveness for their respective “Juan de la Cierva-Incorporación” and “Juan de la Cierva-Formación” postdoctoral contracts (IJCI-2014-19538 and JCFI-2015-23543). We thank Dr. J.M. Igual for his collaboration in the PFLA analysis and J.M. Ordax for his technical assistance.

Appendix A. Supplementary data

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

References

Abdelhafid, R., Houot, S., Barriuso, E., 2000. How increasing availabilities of carbon and nitrogen affect atrazine behaviour in soils. Biol. Fertil. Soils 30, 333–340.
Alonso, D.G., Oliveira Jr., R.S., Hall, K.E., Koskinen, W.C., Constantin, J., Mislankar, S., 2015. Changes in sorption of indaziflam and three transformation products in soil with aging. Geoderma 239–240, 250–256.
Álvarez-Martín, A., Sánchez-Martín, M.J., Pose-Juan, E., Rodríguez-Cruz, M.S., 2016. Effect of different rates of spent mushroom substrate on the dissipation and bioavailability of cymoxanil and tebuconazole in an agricultural soil. Sci. Total Environ. 550, 495–503.
Barriuso, E., Benoit, P., Dubus, I.G., 2008. Formation of pesticide nonextractable (bound) residues in soil: magnitude, controlling factors and reversibility. Environ. Sci. Technol. 42, 1845–1854.
Bhowmick, S., Das, R., Das, A.C., 2014. Effect of thiobencarb and pretilachlor on microorganisms in relation to mineralization of C and N in the Gangetic alluvial soil of West Bengal. Environ. Monit. Assess. 186, 6849–6856.
Botterweck, J., Claßen, D., Zegarski, T., Gottfroh, C., Kalathoor, R., Schäffer, A., Schwarzbauer, J., Schmidt, B., 2014. A correlation between the fate and non-extractable residue formation of 14C-metalaxyl and enzymatic activities in soil. J. Environ. Sci. Health B 49, 69–78.
Braun, K.E., Luks, A.-K., Schmidt, B., 2017. Fate of the ^{14}C-labeled herbicide prosulfocarb in a soil and in a sediment-water system. J. Environ. Sci. Heal. Part B 52, 122–130.
Burauel, P., Führ, F., 2000. Formation and long-term fate of non-extractable residues in outdoor lysimeter studies. Environ. Pollut. 108, 45–52.
Carlsen, S.C.K., Spliid, N.H., Svensmark, B., 2006. Drift of 10 herbicides after tractor spray application. 1. Secondary drift (evaporation). Chemosphere 64, 787–794.
Das, A.C., Barman, S., Das, R., 2015. Effect of pre-emergence herbicides on microbial biomass and biochemical processes in a Typic Fluvaquent soil amended with farm yard manure. Bull. Environ. Contam. Toxicol. 95, 395–400.
EFSA (European Food Safety Authority), 2007. Conclusion on the peer review of prosulfocarb. EFSA Scientific Report 111, 1–81. https://doi.org/10.2903/j.efsa.2007.111r.
Ekler, Z., 1988. Behaviour of thiocarbamate herbicides in soils: adsorption and volatilization. Pestic. Sci. 22, 145–157.
Ferreira Mendes, K., Nogueira de Sousa, R., Takeshita, V., Gimenes Alonso, F., Justiniano Rego, A.P., Luiz Tornisielo, V., 2019. Cow bone char as a sorbent to increase sorption and decrease mobility of hexazinone, metribuzin, and quinclorac in soil. Geoderma 343, 40–49.
FOCUS, 2006. Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. Report of the FOCUS Work Group on degradation kinetics. EC Document Reference Sanco/10058/2005. version 2.0.
García-Delgado, C., Barba, V., Marín-Benito, J.M., Igual, J.M., Sánchez-Martín, M.J., Rodríguez-Cruz, M.S., 2018. Simultaneous application of two herbicides and green compost in a field experiment: implications on soil microbial community. Appl. Soil Ecol. 127, 30–40.
García-Delgado, C., Barba, V., Marín-Benito, J.M., Igual, J.M., Sánchez-Martín, M.J., Rodríguez-Cruz, M.S., 2019. Influence of different agricultural management practices on soil microbial community over dissipation time of two herbicides. Sci. Total Environ 646, 1478–1488.
Gennari, M., Ferraris, L., Nègre, M., Ambrosoli, R., 1998. Behaviour of prosulfocarb in three Italian soils. Fresenius Environ. Bull. 7, 688–694.
Gennari, M., Ambrosoli, R., Nègre, M., Minati, J.L., 2002. Bioavailability and biodegradation of prosulfocarb in soil. J. Environ. Sci. Health B 37, 297–305.
Gevao, B., Jones, K.C., Semple, K.T., Craven, A., Burauel, P., 2003. Nonextractable pesticide residues in soil. Environ. Sci. Technol. 37, 138A–144A.
Guo, L., Jury, W.A., Wagenet, R.J., Flury, M., 2000. Dependence of pesticide degradation on sorption: nonequilibrium model and application to soil reactors. J. Contam. Hydrol. 43, 45–62.
Jablonowski, N.D., Borchard, N., Zajkoska, P., Fernández-Bayo, J.D., Martinazzo, R., Berns, A.E., Burauel, P., 2013. Biochar mediated [^{14}C]atrazine mineralization in atrazine-adapted soils from Belgium and Brazil. J. Agric. Food Chem. 61, 512–516.
Jaquet, J., Weitzel, P., Junge, T., Schmidt, B., 2014. Metabolic fate of the ^{14}C-labeled herbicide clodinafop-propargyl in soil. J.Environ. Sci. Health B 49, 245–254.
Kästner, M., Nowak, K.M., Miltner, A., Trapp, S., Schäffer, A., 2014. Classification and modelling of nonextractable residue (NER) formation of xenobiotics in soil – a synthesis. Crit. Rev. Environ. Sci. Technol. 44, 2107–2171.
Kreuger, J., Paulsson, E., Jonsson, O., 2017. Long-term Monitoring of Pesticides in Air and Atmospheric Deposition in Sweden. Pesticide Behavior in Soil, Water and Air, York, UK.
Marín-Benito, J.M., Andrades, M.S., Sánchez-Martín, M.J., Rodríguez-Cruz, M.S., 2012. Dissipation of fungicides in a vineyard soil amended with different spent mushroom substrates. J. Agric. Food Chem 60, 6936–6945.
Marín Benito, J.M., Herrero-Hernández, E., Andrades, M.S., Sánchez-Martín, M.J., Rodríguez-Cruz, M.S., 2014. Effect of different organic amendments on the dissipation of linuron, diazinon and myclobutanil in an agricultural soil incubated for different time periods. Sci. Total Environ. 476–477, 611–621.
Marín-Benito, J.M., Herrero-Hernández, E., Rodríguez-Cruz, M.S., Arienzo, M., Sánchez-

Martín, M.J., 2017. Study of processes influencing bioavailability of pesticides in wood-soil systems: effect of different factors. Ecotoxicol. Environ. Saf. 139, 454–462.
Marín-Benito, J.M., Barba, V., Ordax, J.M., Andrades, M.S., Sánchez-Martín, M.J., Rodríguez-Cruz, M.S., 2018a. Application of green compost as amendment in an agricultural soil: effect on the behaviour of triasulfuron and prosulfocarb under field conditions. J. Environ. Manag. 207, 180–191.
Marín-Benito, J.M., Barba, V., Ordax, J.M., Sánchez-Martín, M.J., Rodríguez-Cruz, M.S., 2018b. Recycling organic residues in soils as amendments: effect on the mobility of two herbicides under different management practices. J. Environ. Manag. 224, 172–181.
Merlin, C., Devers, M., Béguet, J., Boggio, B., Rouard, N., Martin-Laurent, F., 2016. Evaluation of the ecotoxicological impact of the organochlorine chlordecone on soil microbial community structure, abundance, and function. Environ. Sci. Pollut. Res. 23, 4185–4198.
Nègre, M., Passarella, I., Boursier, C., Mozzetti, C., Gennari, M., 2006. Evaluation of the bioavailability of the herbicide prosulfocarb through adsorption on soils and model soil colloids, and through a simple bioassay. Pest Manag. Sci. 62, 957–964.
Nunes, O.C., Lopes, A.R., Manaia, C.M., 2013. Microbial degradation of the herbicide molinate by defined cultures and in the environment. Appl. Microbiol. Biotechnol. 97, 10275–10291.
Pose-Juan, E., Igual, J.M., Sánchez-Martín, M.J., Rodríguez-Cruz, M.S., 2017. Influence of herbicide triasulfuron on soil microbial community in an unamended soil and a soil amended with organic residues. Front. Microbiol. 8, 378.
Pose-Juan, E., Marín-Benito, J.M., Sánchez-Martín, M.J., Rodríguez-Cruz, M.S., 2018. Dissipation of herbicides after repeated application in soils amended with green compost and sewage sludge. J. Environ. Manag 223, 1068–1077.
PPDB, 2018. Pesticide Properties Data Base. University of Hertfordshire. http://sitem.herts.ac.uk/aeru/ppdb/en/index.htm.
Reid, B.J., Fervor, T.R., Semple, K.T., 2002. Induction of PAH-catabolism in mushroom compost and its use in the biodegradation of soil-associated phenanthrene. Environ. Pollut. 118, 65–73.
Reid, B.J., Pickering, F.L., Freddo, A., Whelan, M.J., Coulon, F., 2013. Influence of biochar on isoproturon partitioning and bioaccessibility in soil. Environ. Pollut. 181, 44–50.
Rodríguez-Cruz, M.S., Pose-Juan, E., Marín-Benito, J.M., Igual, J.M., Sánchez-Martín, M.J., 2019. Pethoxamid dissipation and microbial activity and structure in an agricultural soil: effect of herbicide dose and organic residues. Appl. Soil Ecol. 140, 135–143.
Saison, C., Waller, N.J., Kumar, A., Kookana, R.S., 2009. Effects of thiobencarb in combinations with molinate and chlorpyrifos on selected soil microbial processes. J. Environ. Sci. Health B 44, 226–234.
Scherner, A., Schreiber, F., Fomsgaard, I.S., Melander, B., Avila, L.A., Kudsk, P., 2018. Effect of tillage systems on the dissipation of prosulfocarb herbicide. Weed Technol 32, 195–204.
Sukul, P., Zühlke, S., Lamshöft, M., Rosales-Conrado, N., Spiteller, M., 2010. Dissipation and metabolism of ^{14}C-spiroxamine in soil under laboratory condition. Environ. Pollut. 158, 1542–1550.
Sun, D., Li, K., Bi, Q., Zhu, J., Zhang, Q., Jin, C., Lu, L., Lin, X., 2017. Effects of organic amendment on soil aggregation and microbial community composition during drying-rewetting alternation. Sci. Total Environ. 574, 735–743.
Tabatabai, M.A., 1994. Soil enzymes. In: Weaver, R.W., Angle, J.S., Bottomley, P.S. (Eds.), Methods of Soil Analysis: Part 2. Microbiological and Biochemical Properties. Soil Science Society of America (SSSA), Madison, USA, pp. 903–947.
Wolters, A., Linnemann, V., Herbst, M., Klein, M., Schäffer, A., Vereecken, H., 2003. Pesticide volatilization from soil: lysimeter measurements versus predictions of European registration models. J. Environ. Qual. 32, 1183–1193.
Yuan, Y., Weitzel, P., Schäffer, A., Schmidt, B., 2015. Metabolic fate of the ^{14}C-labeled herbicide clodinafop-propargyl in a sediment–water system. J. Environ. Sci. Health B 50, 533–543.
Zornoza, R., Acosta, J.A., Faz, A., Bååth, E., 2016. Microbial growth and community structure in acid mine soils after addition of different amendments for soil reclamation. Geoderma 272, 64–72.
Zelles, L., 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biol. Fertil. Soils 29, 111–129.