Effect of different organic amendments on the dissipation of linuron, diazinon and myclobutanil in an agricultural soil incubated for different time periods

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ABSTRACT

Dissipation kinetics of pesticides belonging to three chemical groups (linuron, diazinon and myclobutanil) was studied in an unamended agricultural soil and in this soil amended with three organic residues: sewage sludge (SS), grape marc (GM) and spent mushroom substrate (SMS). The soils were incubated with the residues outdoors for one and 12 months. Mineralized, extracted and non-extractable fractions were also studied for ^{14}C-linuron and ^{14}C-diazinon. The dissipation kinetics was fitted to single first-order or first-order multicompartment models. The dissipation rate (k) decreased in the order diazinon > linuron > myclobutanil, and DT_{50} values decreased for linuron (1.6-4.8 times) or increased for myclobutanil (1.7-2.6 times) and diazinon (1.8-2.3 times) in the amended soils relative to the unamended soil. The lowest DT_{50} values for the three pesticides were recorded in GM-amended soil, and the highest values in SMS-amended soil. After 12 months of soil incubation, DT_{50} values decreased in both the unamended and amended soils for linuron, but increased for the unamended and SMS-amended soil for diazinon and myclobutanil. A certain relationship was observed between the sorption of pesticides by the soils and DT_{50} values, although was significant only for myclobutanil ($p<0.05$). Dissipation mechanism recorded the lowest mineralization of ^{14}C-pesticides in the GM-soil despite the highest dissipation rate in this soil. The extracted ^{14}C-residues decreased with incubation time, with increased formation of non-extractable residues, higher in amended soils relative to the unamended soil. Soil dehydrogenase activity was, in general, stimulated by the addition of the organic amendments and pesticides to the soil after one month and 12 months of incubation. The results obtained revealed that the simultaneous use of amendments and pesticides in soils requires a previous study in order to check the environmental specific persistence of these compounds and their effectiveness in amended soils.

***Keywords*:** Pesticide; Agricultural soil; Organic amendment; Dissipation; Mineralization; Soil activity

1. Introduction

Soil protection is a priority objective in modern agriculture. Agricultural soil is a high value resource, and so its irreversible degradation needs to be avoided to guarantee its fertility and its present and future agronomic value. Accordingly, the application of organic amendments to agricultural land is considered a common soil management practice because it avoids the decline in the organic matter (OM) content of agricultural soils, especially soils with a low OM content (< 2%), such as European semi-arid Mediterranean soils. Moreover, these residues provide both macro- and micronutrients to crops, increase water-holding capacity and porosity, and decrease bulk density, thereby contributing to the improvement of the soil's physical and chemical conditions for plant production (Goss et al., 2013).

The management of different organic residues from urban, agricultural and industrial activities has therefore become a priority in many countries today, and different strategies for recycling such materials as organic amendments have been investigated (Moreno Casco and Moral Herrero, 2008) and controlled to avoid the possible threats and risks to human health that may result from their use, as laid down by current Spanish legislation (MARM, 2009; MPR, 2013).

However, the OM in these residues may interfere with the dynamics of the pesticides applied simultaneously with these residues to increase agricultural production and uphold food quality and protection. Pesticides reach the amended soil either by direct application or by the subsequent wash-off from treated plants, and their interaction with the OM of the residues may modify its behaviour in the soil with

respect to unamended soil (Briceño et al., 2008; Wang et al., 2010; Rojas et al., 2013). Considering that the nature and composition of the OM in residues is different to the OM in natural soil, it is of special interest to know how the sorption-desorption, mobility or dissipation of pesticides is affected by soil amendment with organic residues. Changes in these processes might explain the increasingly frequent presence of residues of these compounds in surface and ground waters in agricultural areas (Herrero-Hernández et al., 2013).

Dissipation of pesticides in amended soils can be decreased by the enhanced sorption of these compounds by the OM of the amendments (Grenni et al., 2009; Marín-Benito et al., 2012a,b; Rodríguez-Cruz et al., 2012b) although an apparent increased dissipation may be also observed if irreversible sorption occurs with formation of bound residues (Alexander, 2000). Furthermore dissipation can be affected if soil microbial activity is stimulated by the addition of organic amendments and pesticide biodegradation is enhanced (Moorman et al., 2001; Kadian et al., 2008). To date, a few studies have studied the influence of selected residues on the degradation and persistence of some pesticides in soils amended (Sánchez et al., 2004; Kadian et al., 2008; Fernández-Bayo et al., 2009; Marín Benito et al., 2012b), but only in some of them the dissipation mechanism has been evaluated.

Our group has conducted a research project designed to clarify some of these unexplored aspects regarding the addition of organic residues to soil as amendments and their influence on the behaviour of pesticides. The organic residues studied were sewage sludge (SS) from municipal wastewater treatment operations and the by-products of other agricultural activities generated by the wine industry (grape marc (GM)) and mushroom farming (spent mushroom substrate (SMS)). They are commonly applied to agricultural land in Spain with or without prior treatment. In previous works, we studied

the adsorption (Rodríguez-Cruz et al., 2012a) and the mobility (Marín-Benito et al., 2013) of linuron, diazinon and myclobutanil, in one-month and 12-month incubated soil amended with SS, GM and SMS. The selected pesticides represent groups of compounds with different chemical structures and widely used in agriculture. They are applied in large amounts to a very wide range of crops to control annual grass and broad-leaved weeds, insects and mites or fungal diseases (Tomlin, 2000). However, no dissipation studies have been conducted in these amended soils, and further research is required to assessment the risk of persistence of these compounds over time and their possible contribution to soil and/or water pollution.

Accordingly the aim of this research was to study the effect of the organic residues - SS, GM and SMS - on the dissipation of linuron, diazinon and myclobutanil in a soil amended with these residues after one month and 12 months of incubation in outdoor conditions. We investigated the following: 1) the dissipation kinetics, metabolite formation, and dissipation mechanism of the pesticides to analyze the effect of pesticide properties and the nature and ageing of organic residues, and 2) the soil dehydrogenase activity as an indicator of the soil microbial activity to analyze the effect of amendments and pesticides had on the microbial community.

2. Materials and methods

2.1. Chemicals

Non-labeled linuron (N´-(3,4-dichlorophenyl)-N-methoxy-N-methylurea) was supplied by Riëdel de Haën (Hannover, Germany) (>99% purity). Non-labeled diazinon (O,O-diethyl O-[6-methyl-2-(1-methylethyl)-4-pyrimidinyl] phosphorothioate) and myclobutanil (α-butyl-α-(4-chlorophenyl)-1-H-1,2,4-triazole-1-propanenitrile) from Pestanal were supplied by Sigma-Aldrich Química SA (Madrid, Spain) (>98% purity).

[Ring-U-^{14}C]-linuron (specific activity 9.62 MBq mg^{-1} and 99.07% purity) was supplied by Institute of Isotopes Co., Ltd., (Budapest, Hungary) and [4-methyl-^{14}C]-diazinon (specific activity 610 MBq g^{-1} and 97% purity) was supplied by International Isotopes (Munich, Germany). Myclobutanil was not available as ^{14}C-labeled compound and it was used in the experiment in non-labeled form. The physicochemical properties and environmental fate parameters of the three pesticides, linuron, diazinon and myclobutanil, are given in Table 1. (Tomlin, 2000; FOOTPRINT, 2011).

Linuron metabolites (N-(3,4-dichlorophenyl)-N´-methylurea, N-(3,4-dichlorophenyl)-N´-methoxyurea, N-(3,4-dichlorophenyl)urea and 3,4-dichloroaniline) were supplied by Hoëchst AG (Germany) and their purity was >99.5%. Diazinon metabolite (2-isopropyl-6-methyl-4-pyrimidinol) from Chem Service was supplied by Sigma-Aldrich Química SA (Madrid, Spain) and its purity was 99.5%. HPLC grade methanol and acetone were supplied by Merck (Germany). 2,3,5-Triphenyltetrazolium chloride (TTC) and 2,3,5-triphenylformazan (TPF) were supplied by Sigma-Aldrich Química SA (Madrid, Spain).

2.2. Organic residues

Sewage sludge (SS) from a domestic waste treatment plant and stabilized by anaerobic digestion was supplied by Aqualia SA (Salamanca, Spain). Grape marc (GM), which is comprised of grape stalks, seeds and skins left after the crushing, draining and pressing stages in wine production, was supplied by San Gabriel winery (Aranda de Duero, Spain). Spent mushroom substrate (SMS) from *Agaricus bisporus* (75%) and *Pleurotus sp.* (25%) cultivation is a pasteurized mixture of cereal straw and poultry litter, ammonium nitrate, urea, and minerals (gypsum and/or calcium carbonate), which was further composted for several weeks under aerobic conditions to obtain a

composted SMS. This residue was supplied by Intraval, Tradebe Environmental Group SL (Pradejón, Spain). SS consists mainly of hydrocarbons, amino-acids, small proteins or lipids, with only a small amount of lignin or cellulose, and GM and SMS include cellulose, hemicellulose and lignin in their composition (EC, 2001; Jin and Kelly, 2009; Paredes et al., 2009).

Some characteristics of these organic residues were determined in samples previously air dried, homogenized and sieved (< 2 mm) (Table 2). The pH was determined in a residue/water suspension (1/2.5 w/v ratio). Organic carbon (OC) content was determined by oxidation (Walkley-Black method). Dissolved organic carbon (DOC) was determined in a suspension of residue (1/100 w/v ratio) in Milli-Q ultrapure water after residue shaking (24 h at 20ºC), centrifugation (20 min at 10000 rpm), and filtering (Minisart NY 25 filter 0.45µm, Sartorius Stedim Biotech, Germany) using a Shimadzu 5050 (Shimadzu, Columbia, MD, USA) organic carbon analyzer.

2.3. Unamended and amended soil

The soil used in all experiments was a Typic Xerorthent sandy loam soil (Soil Survey Staff, 2006) taken from the surface horizon (0-30 cm) of a vineyard farm in the Castilla-Leon region (NW-Spain) located in Pesquera de Duero (41º 38’ 34’’N latitude and 4º 9’ 27’’ W longitude). The soil was sieved (< 2 mm) and dried to determine their characteristics using standard analytical methods (MAPA, 1986). The soil pH determined in a soil/water suspension (1/2.5 w/v) was 7.9. The particle size distribution determined using the pipette method was 76.9%, 8.2% and 14.9% sand, silt and clay, respectively. The OC content determined by oxidation (Walkley-Black method) was 0.47%. The DOC was 0.04 mg g^{-1}, it was determined in soil extracts (1/2 w/v ratio) in Milli-Q ultrapure water after soil shaking (24 h at 20ºC), centrifugation (20 min at

10000 rpm), and filtering (Minisart NY 25 filter 0.45µm, Sartorius Stedim Biotech, Germany) using an organic carbon analyzer as previously indicated for organic residues. Inorganic carbon content determined as $CaCO_3$ with a Bernard calcimeter was 2.25%.

The amended soils were prepared by uniformly mixing soil with SS, GM or SMS at a rate of 5% on a dry weight basis (equivalent to ~25 t residue ha^{-1} considering a soil depth of ~5 cm and a soil density of 1.3 g cm^{-3}) on 15 November 2009. Soil and organic residues were mixed without sieving (undisturbed).

Samples of all treatments (unamended and amended soil) (~30 kg) were incubated under environmental conditions in 60 x 40 x 25 cm trays in the IRNASA (Salamanca, Spain) over 12 months. The initial moisture content of the unamended and amended soil was previously adjusted to 40% of their maximum water holding capacity. Weather conditions were recorded throughout the experiment. The monthly average minimum air temperature varied between -0.3 and 13.3ºC, whilst the monthly average maximum air temperature varied between 8.5 and 32.7ºC. Cumulative precipitation during the experiment was 426 mm.

Samples of unamended soil and soil amended with different residues were taken after one month (one-month incubated soils) and 12 months (12-month incubated soils) of incubation outdoors. They were sieved (<2 mm) and dried and the OC and DOC contents and pH values were determined as previously indicated. In addition, alkali soluble and acid insoluble carbon (humic acid, HA) and alkali and acid soluble carbon (fulvic acid, FA) were also determined in unamended and amended soils. Soil extracts were obtained following the traditional method of HA and FA extraction using 5 g of soil and an initial solution of Na_4PO_7·and Na_2SO_4·in H_2SO_4 (25 mL) (Gallardo and Bacas, 1973). The determination of C in solution was carried out as previously described for the DOC. Results obtained are included in Table 2.

Changes in the DOC contents and the ratio HA/FA determined in amended soils after one month and 12 months of incubation were related to the evolution of OC of amended soils over time.

2.4. Dissipation studies

Initially, pesticides were individually dissolved in methanol to give a concentration of 1000 mg L^{-1}. Solutions of each pesticide were then prepared in Milli-Q ultrapure water, and a volume of 10 mL of appropriate concentration was added to 300 g of fresh weight of unamended or amended soil taken from the trays and sieved (<2 mm) to give a concentration of 2 mg kg^{-1} dry soil. Samples of soil+pesticide were incubated in the dark in loosely capped appropriate containers at 20 ^{o}C in a thermostated chamber. The moisture content of soil samples was previously adjusted to 40 % of the maximum soil water-holding capacity, and it was maintained by adding sterile Milli-Q ultrapure water when necessary. Each soil treatment was prepared in duplicate.

A sterilized soil sample was also prepared by autoclaving soil in erlenmeyer flasks at 120 ^{o}C for 1 h on three consecutive days. Sterilized unamended soil was treated with each pesticide and incubated as indicated above, and these samples were used as controls to check the chemical degradation of pesticides.

Finally, soils for microbiological control were prepared by adding only sterile Milli-Q ultrapure water. All soils were thoroughly stirred with a sterilized spatula, and all of the steps were performed in a sterile cabinet. Soil samples were taken at day 0 for fungicide analysis, and thereafter repeatedly at different time intervals (up to 147 days) depending on the dissipation rate of each pesticide.

2.5. Extraction and determination of pesticides

Duplicate 5 g samples of each duplicate treatment (300 g of unamended or amended soil treated with different pesticides) were taken at each sampling time and shaken at 20 ^{o}C for 24 h with 10 mL of methanol (linuron and diazinon) or acetone (myclobutanil) in glass tubes. The samples were then centrifuged at 5045 g for 15 min, and the pesticide extracts were filtered in a Minisart NY 25 filter (Sartorius Stedim Biotech, Germany) to remove particles >0.45 µm. For the determination of the unlabeled pesticides and their metabolites, a volume of the extract (6 mL) was transferred to a clean glass tube and evaporated at 25 ^{o}C under a nitrogen stream using an EVA-EC2-L evaporator (VLM GmbH, Bielefeld, Germany) until dryness. The residue was dissolved in 0.75 mL of methanol and transferred to a glass vial for analysis. The recoveries of the extraction method were determined by spiking three unamended and amended soil samples with analytical grade pesticide to a final concentration of 2 mg kg^{-1} and performing the extraction procedure as described above. The mean recovery values were >90 %, >80 % and >85 % for linuron, diazinon and myclobutanil, respectively.

Linuron, diazinon, and myclobutanil were quantified by HPLC with diode array (DAD) and mass spectrometer (MS) detectors (Waters Associates, Milford, MA), using a Waters Symmetry C18 column (75 × 4.6 mm i.d., 3.5 µm) at ambient temperature. The mobile phase was 72:28 (v/v) methanol/ammonium formate 5 mM for linuron, 80:20 (v/v) methanol/water for diazinon, and 75:25 methanol/water (0.1 % formic acid) for myclobutanil. The flow rate of the mobile phase was 0.3 mL min^{-1} and the sample injection volume was 10 µL. The retention time was 6.8, 7.2, and 6.3 min for linuron, diazinon, and myclobutanil. Quantitative analysis was performed using the peak area of each compound obtained from the total ion chromatogram (TIC) in SIM mode. The molecular ions (m/z) corresponding to each pesticide in the positive ionization mode

$[M]^+$ were 250.1, 305.2, and 289.1 for linuron, diazinon, and myclobutanil, respectively. Calibration was performed from 0.05 to 2.5 µg mL^{-1} and the limit of detection (LOD) and limit of quantification were >0.01 and >0.03 µg mL^{-1}, respectively, for all of the pesticides.

Monitoring also involved positive molecular ions (m/z) 220.1, 236.1, 206.1 and 162.0 for linuron metabolites (N-(3,4-dichlorophenyl)-N´-methylurea, N-(3,4-dichlorophenyl)-N´-methoxyurea, N-(3,4-dichlorophenyl)urea and 3,4-dichloroaniline), 153.2 for diazinon metabolite (2-isopropyl-6-methyl-4-pyrimidinol), and 128.1 for myclobutanil metabolite (1-H-1,2,4-triazol-1-ylacetic acid). The myclobutanil metabolite was only qualitatively monitored.

2.6. Mineralization and mass balance of ^{14}C-linuron and ^{14}C-diazinon

For linuron and diazinon, simultaneous incubations were carried out with ^{14}C-labeled pesticides to study the dissipation mechanism (mineralization kinetics and the formation of non-extractable residues over time). Aqueous solutions of unlabeled pesticide of an appropriate concentration were labeled with ^{14}C-pesticides, and a volume of 10 mL of these solutions was added to 300 g of fresh weight of unamended or amended soils to give a concentration of 2 mg kg^{-1} dry soil and an approximately activity of 100 Bq g^{-1}. In these soil samples, a $^{14}CO_2$ trap, consisting of a scintillation vial containing 1 M NaOH (1 ml), was attached to the lid via a stainless steel clip as described by Reid et al. (2002).

The extraction of the ^{14}C-pesticides from the soil was carried out in two sequential steps: Initially the ^{14}C-pesticide was extracted with 10 mL of a 0.01 M $CaCl_2$ Milli-Q ultrapure water solution for 24 h, and after a second extraction with 10 mL of the organic solvent methanol was carried out for 24 h.

The quantitative determination of ^{14}C-linuron and ^{14}C-diazinon after extraction was performed by liquid scintillation using a Beckman LS6500 liquid scintillation counter (Beckman Instruments Inc., Fullerton, CA, USA). The radioactivity of the solution was measured in disintegrations per minute (dpm), being determined in duplicate in 1 mL of aqueous or methanol extracts to which 4 mL of scintillation cocktail was added (Ecoscint TMA, National Diagnostics, Atlanta, GA, USA). Residues of ^{14}C-pesticides remaining in the soil after extraction were determined by the combustion of triplicate 1 g dried soil samples, using a Biological Oxidizer (R.J. Harvey OX-500 Instrument Corp., NJ) under O_2 excess at 900 ^{o}C. The $^{14}CO_2$ generated was trapped in a mixture of ethanolamine (1 mL) and scintillation cocktail (Oxisolve C-400, Zinsser Analytic, Berkshire, UK; 15 mL) and determined as indicated above.

$^{14}CO_2$ from mineralized ^{14}C-pesticides in the scintillation vial containing 1 M NaOH (1 mL) was determined at the different sampling times by mixing with 4 mL of scintillation cocktail and determined as previously indicated.

2.7. Soil dehydrogenase activity

Soil dehydrogenase activity (DHA) was determined following the Tabatabai method (Tabatabai, 1994) at the beginning and at the end of the dissipation period. The method is based on the extraction and colorimetric determination of the intensely colored TPF produced from the reduction of colorless TTC in soils.

2.8. Data analysis

The dissipation kinetics for the pesticide 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. C is the pesticide

concentration at time t, C_0 is the initial pesticide concentration, k (day^{-1}) is the dissipation rate, α is a shape parameter determined by the coefficient of variation of k values and β is a location parameter. For the selection of the kinetic model that best describes the dissipation results, FOCUS work group guidance recommendations were followed (FOCUS, 2006) 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 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 dissipaion 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 treatments on the dissipation of pesticides. 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. Statgraphics Plus version 5.1 statistical software (Statgraphics Plus Corp., Princeton, NJ, USA) was used.

3. Results and discussion

3.1. Dissipation kinetics of pesticides in the unamended and amended soil

Figure 1 shows the decrease in the concentrations of non-labeled pesticides (expressed as a percentage of the amount of pesticide initially applied) in the unamended and amended soils after incubation for one month and 12 months. The study

was conducted until the dissipation of linuron, diazinon and myclobutanil fell into the ranges of 57-98%, 77-97% and 56-87%, respectively, which occurred after 62 days (linuron), 35 days (diazinon), and 147 days (myclobutanil) of incubation of the pesticide in the soils. The data for the residual concentrations of pesticides as a function of time were fitted to SFO and FOMC models, and the kinetic parameters were calculated for each pesticide, soil treatment and soil incubation period (Table 3).

Linuron dissipation kinetics fitted the SFO model better than the FOMC model (χ^2 error values were lower than those corresponding to the FOMC model), as also indicated in previous studies (Rodríguez-Cruz et al., 2001; Grenni et al., 2009) for the dissipation of this herbicide in an unamended soil or that amended with different organic materials. The dissipation kinetics of linuron in some samples (one-month incubated unamended and SMS-amended soil) initially recorded a lag phase of 13 days with no dissipation, followed by a rapid dissipation phase that closely fitted a SFO model (Figure 1). The existence of a lag phase has also been observed in the dissipation kinetics of other pesticides (Marín-Benito et al., 2012b), and it reflects the adaptation time needed for the microbial community to degrade the pesticide.

The dissipation kinetics of diazinon also fitted the SFO model in the unamended soil and the SS-amended soil after one month and 12 months of incubation. However, diazinon dissipation fitted the FOMC model better in the GM- and SMS-amended soil for both incubation times. Diazinon dissipation initially recorded a lag phase of 16 days (Soil+SS) or 16-20 days (Soil+SMS), and it was followed by a rapid pesticide dissipation phase. Different models with or without lag phase have also been reported in the literature to fit the dissipation curves of diazinon in unamended soils and soils treated with municipal waste water and surfactant solutions (Hernández-Soriano et al., 2009; Cycon et al., 2010a).

For myclobutanil, in general, the dissipation kinetics fitted the FOMC model better than the SFO model in one-month incubated soils and the SFO model in 12-month incubated soils, although the literature reports that the dissipation curves of myclobutanil in unamended soil usually fitted a SFO model (Wang et al., 2012). All the dissipation kinetics of myclobutanil recorded a lag phase in a range of 8-28 days, and the duration of the lag phase was shorter in the 12-month incubated soils (Figure 1).

The dissipation rate (k) decreased in the order diazinon > linuron > myclobutanil in the unamended soil. DT_{50} values in days for the dissipation of the pesticides studied were 49.8 and 21.2 (linuron), 10.1 and 20.2 (diazinon) and 48.9 and 65.6 (myclobutanil) in the unamended soil after one month and 12 months of incubation. Changes in the DT_{50} values of pesticides were significant (Table 3) after unamended soil ageing, although changes in the soil characteristics (OM, sorption parameters, etc.) were not significant. These results are in agreement with the wide range of DT_{50} values found in the literature (Table 1) for the dissipation of these pesticides in different unamended soils (FOOTPRINT, 2011).

After soil amendment, the dissipation of diazinon and myclobutanil was slower in the SS- and SMS-amended soil than in the unamended one. Note should be taken of the existence of a lag phase before a rapid dissipation phase in these soils. This effect was expected, given the increase in the sorption of both pesticides by amended soils relative to the unamended one and the influence of sorption on the dissipation kinetics of pesticides in soils due to a decrease in the bioavailability and biodegradation of organic compounds sorbed by the soil (Alexander, 2000). DT_{50} values increased 1.7 and 2.6 times for myclobutanil, and 2.3 and 1.8 times for diazinon in the SS- and SMS-amended soil, respectively. However, the dissipation in GM-amended soil was similar

for myclobutanil or increased for diazinon (DT_{50} values decreased 1.8 times relative to the unamended soil).

An opposite effect was observed for the dissipation of linuron; it was more rapid in the amended soils than in the unamended one, although the sorption of linuron by the amended soils increased (Table 1). DT_{50} values decreased between 1.6 and 4.8 times in the amended soils. According to these results, it may be assumed that different mechanisms are involved in the dissipation of pesticides in amended soils.

Regarding the three pesticides, the lowest DT_{50} values were generally obtained in the GM-amended soil, with the highest in the SMS-amended soil (Table 3). The GM-amended soil has the highest DOC content (Table 2), and the pesticides could be sorbed by the DOC in soil solution, to a greater or lesser extent increasing their availability for degradation (Barriuso et al., 2011). The highest DT_{50} values of linuron and myclobutanil in the SMS-amended soil could be explained by the high sorption of these pesticides by this soil (Table 1) with more stabilized OM, taking into account that the HA/FA ratio was the highest among the amended soils (Table 2).

DT_{50} values decreased for linuron in the SS- and SMS-amended soil after 12 months of incubation, and increased for diazinon and myclobutanil in the SMS-amended soil with regard to the corresponding soil after one month of incubation (Table 3). No significant differences were found in the DT_{50} values for pesticides in the GM-amended soil. Moreover, for diazinon, dissipation was more rapid in the SS-amended soil due to the suppression of the lag phase recorded in the homologous one-month incubated soil. The lag phase was also shorter for the dissipation of myclobutanil in the 12-month incubated soils than in the one-month incubated soil. This indicated that the adaptation time needed for the microbial community to degrade myclobutanil and

diazinon in some amended soils was shorter in the 12-month incubated soils, possibly due to the decrease in OC and DOC in these soils (Table 2).

Dissipation could be related to the sorption behaviour of the studied pesticides by soils, in keeping with the decrease or increase in their sorption by the amended soils after one-month and 12-month ageing, as discussed by the authors in a previous work (Rodriguez-Cruz et al., 2012a). However, the sorption coefficients of linuron and diazinon by soils (Table 1) were not significantly correlated with the DT_{50} values when one-month and 12-month incubated soils were considered in the analysis. A positive and significant correlation ($r=0.79$, $p<0.05$) between the sorption coefficients and DT_{50} values was found solely for myclobutanil when one-month and 12-month incubated soils were considered together.

The influence of sorption on the degradation kinetics of some fungicides in different SMS-amended soils has been observed in previous studies (Marín-Benito et al., 2012b). Regarding the pesticides studied here, Rodríguez-Cruz et al. (2001) have reported a decrease in the degradation rate of linuron in soils with a liquid humic amendment and peat due to herbicide adsorption by amended soils. However, the degradation of linuron in city refuse compost-amended soil increased due to an increase in the microbial activity after soil amendment. The linuron half-life values indicated a slower degradation rate in pine- and oak-amended soils than in unamended ones (Grenni et al., 2009). A decrease in the dissipation rate of diazinon in soil amended with sewage sludge was observed and attributed to a reduction in its availability and, therefore, a greater persistence compared to the untreated soil (Sánchez et al., 2004). The degradation of diazinon in neem cake-amended soils was prolonged compared to soils without amendment, increasing the persistence of the insecticide (Akhtar et al., 1998).

There are no previous studies on the influence of sorption on the dissipation of myclobutanil in amended soils.

Results of the dissipation of pesticides in sterilized unamended soil indicated that the soil microbial community played an active role in this process, as no actual dissipation was observed, or it was much slower than for non-sterilized soil (data not shown). However, the dissipation data for linuron in the 12-month incubated sterilized soil (76% remained after 62 days) and diazinon in the one-month incubated sterilized soil (81% remained after 37 days) suggest the influence of other abiotic factors, such as chemical hydrolysis, as previously reported (Rodríguez-Cruz et al., 2001; Sarmah et al., 2009).

To check the possible chemical hydrolysis of pesticides, some metabolites of linuron, diazinon and myclobutanil were monitored during the dissipation study. Myclobutanil metabolites were not detected in the soil extracts, but traces of linuron metabolites were detected in the unamended and amended soils (data not shown). The formation of metabolites might explain the rapid dissipation of linuron in the soils and the absence of correlation between DT_{50} values and sorption coefficients. The metabolites formed could be sorbed by soils, as indicated for other compounds in amended soils (Marín-Benito et al., 2012b).

A diazinon metabolite (2-isopropyl-6-methyl-4-pyrimidinol (IMP)) was detected in the soil extracts from the one-month and 12-month incubated soils (Figure 2). IMP is a diazinon hydrolysis product (Bavcon et al., 2003). The higher amount of IMP was detected in the unamended soil between 16 and 20 days (21%, expressed as a percentage of the diazinon initially applied). In the amended soils, the maximum amounts of IMP detected were 3.1-9.5% between 7 and 28 days in the one-month incubated soils and 5.1-15.3% between 8 and 20 days in the 12-month incubated soils.

In the amended soil, the percentages of IMP increased in the order: soil+SMS< soil+SS< soil+GM after both incubation periods. These results were consistent with the rapid dissipation rate of diazinon in the unamended soil and in the GM-amended soil. The formation of IMP in unamended soils has been reported previously (NRA, 2002; Bavcon et al., 2003; Leland et al., 2003).

3.2. Mass balance of ^{14}C-linuron and ^{14}C-diazinon in the unamended and amended soil

The total ^{14}C balance corresponding to mineralized, extracted (as parent or metabolites), and non-extractable (bound residues) ^{14}C-linuron and ^{14}C-diazinon was determined to explain dissipation mechanism in the unamended and amended soil incubated for one month (Figure 3) and in unamended and amended soil incubated for 12 months (data not-shown). The total mass balance (expressed as a percentage of the ^{14}C initially applied) was, in general, >84% for ^{14}C-linuron (79-95% range) and >80% for ^{14}C-diazinon (70-104% range).

The mineralization of linuron was lower than that of diazinon, and followed the same pattern for both pesticides in the one-month and 12-month incubated soil, although the total percentage of mineralization varied between the soils for different incubation times.

Linuron mineralization was low in all the soils and increased slowly over the incubation period. The amounts of ^{14}C-linuron mineralized to $^{14}CO_2$ after 83 days were 2.45%, 1.16%, 0.38% and 0.67%, in unamended soil, soil+SS, soil+GM and soil+SMS, respectively, after one month of incubation, and 2.14%, 1.15%, 0.92% and 1.62%, in the respective unamended and amended soils after 12 months of incubation. The lower values were recorded in the amended soils, even though the dissipation rates were

higher in these soils than in the unamended one. As previously indicated, this reveals that dissipation in amended soils must occur via another mechanism derived from the possible sorption of linuron or its metabolites, although other authors have also attributed the decrease of mineralized pesticide to the use of the OM added with the amendment by the microorganisms instead of the pesticide (Fernandes et al., 2006).

Mineralization was initially very slow for diazinon in the unamended and amended soils, although a higher increase in $^{14}CO_2$ evolution was observed in the SS- and SMS-amended soil over time. This low initial mineralization must correspond to the previous adaptation period of the soil microbial community, according to the lag phase observed in some of the amended soils (Figure 1), and biodegradation could then occur more quickly. The percentages mineralized after 35 days were 8.51%, 6.21%, 1.09% and 6.74%, in unamended soil, and SS-, GM- and SMS-soil, respectively, after one month of incubation. These amounts were 5.45%, 7.98%, 4.22% and 6.68% in 12-month incubated soils. ^{14}C-Diazinon mineralization in all the soils was higher than that of linuron, possibly because the ^{14}C-label of diazinon is located in the side methyl group, compared to the ^{14}C-label of linuron on the ring, and may therefore allow a greater production of $^{14}CO_2$. The lower mineralization was recorded in the unamended soil and GM-amended soil despite the highest dissipation rates found in these soils. This is consistent with the formation of a high amount of metabolite IMP in these soils (Figure 2), which must be degraded to $^{14}CO_2$ more slowly, as mineralization kinetics indicates. In relation to this, a report drafted by the National Registration Authority of Australia (NRA, 2002) has indicated that IMP is slowly degraded and mineralized to $^{14}CO_2$ in soils.

The extracted amounts in $CaCl_2$ and methanol decreased for both pesticides as incubation time increased. In general, the decrease was more rapid in the amended soils with respect to the unamended one after both soil incubation periods (Figures 3).

Soil extraction with water solutions (0.01M $CaCl_2$) provides an estimate of the availability of pesticide residues to be degraded, and depends mainly on pesticide sorption (Mamy et al., 2005). For linuron the ^{14}C amounts in water extracts were initially higher in the unamended soil (up to 27%) than in the amended ones (up to 18%), although they were similar in both one-month and 12-month incubated soils (Figure 3). These amounts were time dependent and decreased quickly after 83 days in the unamended (< 3%) and amended soils (<2%) incubated for one and 12 months. These results are consistent with the linuron sorption coefficients (Table 1), which were higher for the amended soils.

However, the amounts of ^{14}C-linuron extractable in methanol were initially very similar in the unamended and amended soils (55-68%), but the decrease was higher in the amended soils with respect to the unamended soil as indicated for non-labeled pesticides (Figure 3). The extracted ^{14}C amounts were 19.4% (unamended soil) and 11.7%, 10.2%, and 9.48% (SS-, GM-, and SMS-amended soil) of the ^{14}C-linuron initially added after 83 days in one-month incubated soils, and 15.6% (unamended soil) and 10.3%, 9.50%, and 9.98% (SS-, GM-, and SMS-amended soil) after 83 days in 12-month incubated soils. The higher amount of extracted pesticide in the unamended soil with regard to the amended soils was consistent with the slowest dissipation rate in this soil.

The water extractable residues for diazinon were initially much higher than for linuron according to its highest rate of dissipation, and they decreased with incubation time. ^{14}C amounts in water extracts were higher than 50% at the beginning of the

incubation period in the unamended and amended soils in both one-month and 12-month incubated soils (Figure 3). These amounts decreased rapidly in the SS-amended and SMS-amended soils up to <10%, but changes in the extracted amounts in the unamended and GM-amended soils were smaller, and they were still high after 35 days, 26-37% in one-month incubated soils, and 22-25% in 12-month incubated soils (Figure 3).

The methanol extracts for diazinon were initially similar (33-44%) in the unamended and amended soils, and decreased more quickly in the SS-amended and SMS-amended soil than in the GM-amended and unamended soils. After 35 days, these amounts were 8.01-6.60% (unamended soil), 5.79-5.67%, 14.1-11.8%, and 4.70-8.95% (SS-, GM-, and SMS-amended soil) of the ^{14}C-pesticide added in the one-month and 12-month incubated soils. The extracted amounts of ^{14}C corresponded to the parent compound and its metabolites formed during degradation, and they were consistent with the higher formation of the IMP metabolite in the unamended soil and the GM-amended soil (Figure 2), which could be extracted with the organic solvent.

For both pesticides the amounts of non-extractable residues increased with the longer pesticide incubation time in the soils, as reported previously for other compounds (Fenlon et al., 2011; Marín-Benito et al., 2012b). The percentages of non-extractable residues of linuron formed at the end of the incubation time of 83 days were 35.8% (unamended soil) and 69.1%, 79.9% and 63.1% (SS-, GM-, and SMS-amended soil) in one-month incubated soil and 66.5% (unamended soil) and 72.4%, 82.7%, and 82.3% (SS-, GM-, and SMS-amended soil) after 83 days in the 12-month incubated soils. For diazinon, these percentages were 55.6-30.7% (unamended soil), 47.3-52.4, 35.0-51.8%, and 57.0-51.9% (SS-, GM-, and SMS-amended soil) after 35 days of incubation in one-month and 12-month incubated soils, respectively.

The formation of non-extractable residues in the soils was in general higher for linuron than for diazinon, possibly0 due to the higher sorption of linuron by soils than of diazinon (Table 1). The formation of bound residues for linuron was higher in the amended soils in relation to the unamended soil in the one-month incubated soils. This is consistent with the more rapid dissipation relative to the unamended soil (Table 3), as the formation of non-extractable residues leads to a decrease in availability and an apparent increase in the dissipation rate. This effect is less significant in the 12-month incubated soils, and dissipation rates in these soils were in closer agreement with the decreased sorption of linuron by the amended soils after 12 months of incubation (Table 1). The bound residues could be forthcoming from the parent compounds or metabolites of pesticides according to the results recorded in the GM-amended soil, where the pesticide could be sorbed by DOM, enhancing the formation of metabolites with a higher sorption capacity by soils.

For diazinon, the highest initial formation of non-extractable residues in the SMS-amended soil is consistent with the low formation in it of the IMP metabolite. The growing amounts of non-extractable residues built up over time must also correspond to both ^{14}C-pesticide and ^{14}C-metabolites. In a previous paper, Leland et al. (2003) have reported that diazinon and its metabolite IMP are associated with either the humic or the humic substance fractions in a sorbed state within micropores or intraparticle nanopores. Accordingly, some authors contend that soil OC content is the key factor involved in the formation of non-extractable residues (Mamy et al., 2005).

3.3. Soil dehydrogenase activity

Dehydrogenase activity (DHA) was determined as an indicator of the soil microbial activity for the unamended and amended soils, either untreated (controls) or

treated with pesticides at the beginning and at the end of the dissipation of pesticides in one-month and 12-month incubated soils. Results obtained are included in Figure 4. In general, mean DHA values were higher in the amended soils than in the unamended one, indicating the positive effect of the amendment on soil microbial activity as reported previously (Fernández-Bayo et al., 2009; Marín-Benito et al., 2012b; Rodríguez-Cruz et al., 2012b). The addition of organic residues to the soil stimulated DHA due to the greater OC content available in the amended soil and the presence of new soil microbial populations introduced with the amendment. Furthermore, DHA was higher in the soil+GM, due to its higher OC content and the degradation of more labile compounds provided by the amendment as DOC. The higher DHA in this soil is also consistent with the higher degradation rate of pesticides when compared with the soil+SS or soil+SMS. DHA values in controls (soil without pesticide) at the beginning of the dissipation study were similar (unamended soil) or decreased (amended soils) relative to DHA values at the end of the pesticide dissipation (62, 35 and 147 days). The decrease in DHA values for the 12-month incubated soils was greater than for the one-month incubated soils.

DHA values in the unamended and amended soils after one month and 12 months of incubation treated with the pesticides were, in general, higher than in the control soil (without pesticide), indicating that soil microbial activity was stimulated by the addition of pesticides to the soil. In the GM-amended soil treated with linuron, diazinon or myclobutanil, DHA was similar or decreased significantly with respect to the GM-amended soil without pesticide (control soil) at the beginning of the incubation period. The pesticide had a toxic effect on soil microorganisms, and slightly inhibited soil DHA. At the end of the incubation period, the pesticide's effect on soil microbial activity was less marked, suggesting that this impact on soil microorganisms

disappeared when the pesticide was dissipated. A similar effect was observed by Kadian et al. (2012) in unamended and amended soils. However Cycon et al. (2010a) reported a decrease in DHA in soils treated with diazinon indicating this effect might have resulted from the death of a microbial fraction sensitive to the insecticide and the rapid degradation of the enzyme released from cells. Stimulation or inhibition of the DHA in response to soil treated with pesticides has been reported in the literature and these different impacts of pesticides on DHA could be associated with differences in soil characteristics, the composition of microbial communities and the type and dosage of compound (Cycon et al., 2010b).

4. Conclusions

This study revealed a different effect of organic residues (SS, GM and SMS) applied as amendments on the dissipation of pesticides in an amended agricultural soil. Compared to unamended soil the DT_{50} values of pesticides decreased for linuron in all amended soils, and increased for diazinon and myclobutanil in the SS- and SMS-amended soil. The dissipation was higher for all the pesticides in the GM-amended soils, and lower in the SMS-amended soils. The highest DOC content of GM-soil could enhance the sorption by the DOC in soil solution increasing their availability for degradation while the more stabilized OM of SMS-soil could increase the sorption of pesticides. The effect of soil ageing on dissipation was consistent with the changes in the sorption of pesticides by soils after incubation. Different dissipation mechanisms were revealed for ^{14}C-linuron and ^{14}C-diazinon. Mineralized and extractable amounts were higher for diazinon than for linuron. For both pesticides, the extractable amounts decreased and the non-extractable amounts increased as incubation time increased, which is consistent with the dissipation rates. The effect of soil ageing was seen mainly

in the decrease in extractable amounts for diazinon and in the increase in non-extractable amounts for linuron. A positive effect of the amendment and pesticide on soil microbial activity was revealed by DHA values determined in the unamended and amended soils. The results obtained indicated the influence of the nature of organic residue on the dissipation of pesticides in amended soils and they are of interest to improve our knowledge of the persistence of these compounds in soils when amendments and pesticides are simultaneously applied in agricultural practices.

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Figure captions

Fig. 1. Dissipation kinetics of linuron, diazinon and myclobutanil in unamended soil and in soil amended with sewage sludge (SS), grape marc (GM) and spent mushroom substrate (SMS) incubated for one month and 12 months. Bars indicate the standard deviation of the mean.

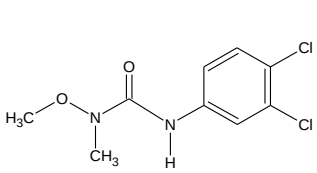
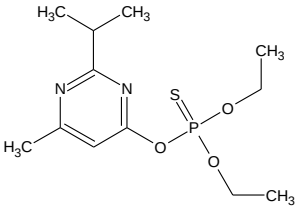
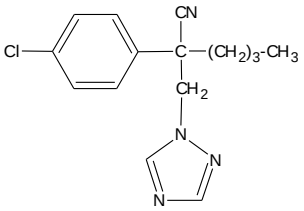
Fig. 2. Diazinon metabolite (IMP) formation over time in unamended soil and in soil amended with sewage sludge (SS), grape marc (GM) and spent mushroom substrate (SMS) incubated for one month and 12 months. Bars indicate the standard deviation of the mean.

Fig. 3. Mass balance of mineralized, $CaCl_2$-extracted, methanol-extracted, and non-extracted ^{14}C (expressed as percentage of applied ^{14}C) for the dissipation studies of ^{14}C-linuron (left side) and ^{14}C-diazinon (right side) in unamended and amended soils incubated for one month

Fig. 4. Soil dehydrogenase activity for unamended and soil amended with sewage sludge (SS), grape marc (GM) and spent mushroom substrate (SMS), incubated for one month and 12 months, untreated and treated with pesticides linuron (LN), diazinon (DZ) and myclobutanil (MB) at the beginning and at the end of the incubation period. Bars indicate the standard deviation of the mean.

Table 1

Physicochemical properties and environmental fate parameters of selected pesticides.

	Linuron	Diazinon	Myclobutanil
Chemical structure			
Solubility in water (mg L^{-1})[a]	63.8	60.0	132
log Kow[a]	3.0	3.3	2.9
Koc (mL g^{-1})[a]	500-620	413-760	225.7-920
DT_{50} (days)[a]	38-87	9.1-21	35-365
GUS index[a]	2.03	1.14	3.20
Kd[b]			
SOIL	1.77 [c] - 2.57 [d]	0.54 [c] - 1.24 [d]	1.35 [c] - 2.93 [d]
SOIL+SS	5.13 [c] - 3.97 [d]	1.67 [c] - 1.46 [d]	4.06 [c] - 8.0 [d]
SOIL+GM	8.53 [c] - 4.59 [d]	3.76 [c] - 2.70 [d]	4.98 [c] - 5.60 [d]
SOIL+SMS	8.23 [c] - 6.78 [d]	1.78 [c] - 1.46 [d]	7.22 [c] - 10.6 [d]

[a] From Tomlin (2000) and FOOPRINT (2011)

[b] Sorption coefficients by [c] one-month and [d] 12-month incubated soils taken from Rodríguez-Cruz et al. (2012a)

Table 2

Selected characteristics of organic residues and unamended soil and amended soil with sewage sludge (SS), grape marc (GM) and spent mushroom substrate (SMS) incubated for one month and 12 months.

Samples	pH	OC %	DOC (mg g^{-1})	HA[a] (mg g^{-1})	FA[b] (mg g^{-1})	HA/FA
SS	6.1	24.8	1.98			
GM	5.0	41.8	5.88			
SMS	7.1	26.7	1.22			
1-month soils						
SOIL	7.7	0.47	0.04	0.37	0.91	0.41
SOIL+SS	7.3	2.01	0.13	0.41	1.56	0.26
SOIL+GM	7.4	2.67	0.81	0.34	2.10	0.16
SOIL+SMS	7.5	2.18	0.36	1.34	1.69	0.79
12-month soils						
SOIL	7.8	0.58	0.01	0.27	0.70	0.39
SOIL+SS	7.2	1.35	0.04	0.42	1.20	0.35
SOIL+GM	7.0	2.03	0.24	0.46	1.50	0.31
SOIL+SMS	7.1	1.95	0.07	1.06	1.10	0.96

[a] HA, alkali soluble organic carbon, [b] FA, acid and alkali soluble organic carbon

Table 3

Kinetics parameters for the dissipation of linuron, diazinon and myclobutanil in unamended soil and amended with sewage sludge (SS), grape marc (GM) and spent mushroom substrate (SMS), incubated for one month and 12 months, obtained from fitting kinetics to a single first-order (SFO) and Gustafson and Holden (FOMC) models.

Samples	Single First Order (SFO)				Gustafson and Holden (FOMC)				
	K (d^{-1})	Lag (d)	DT_{50} [a]±SD (d)	χ^2	α	β	Lag (d)	DT_{50}±SD (d)	χ^2
1-month soil					Linuron				
SOIL	0.019	13	49.8±0.78a	6.4	$2.1x10^4$	$1.1x10^6$	13	49.8±0.78a	6.8
SOIL+SS	0.026		26.7±0.71c	9.9	$3.3x10^4$	$1.3x10^6$		26.7±0.71c	10.5
SOIL+GM	0.066		10.4±0.64g	10.1	$3.8x10^4$	$5.8x10^5$		10.4±0.64g	10.7
SOIL+SMS	0.039	13	30.9±0.28b	8.1	$3.4x10^4$	$8.8x10^5$	13	30.9±0.28b	8.9
12-month soil									
SOIL	0.033		21.2±0.35e	7.7	$4.6x10^4$	$1.4x10^6$		21.2±0.35e	8.1
SOIL+SS	0.035		19.8±0.42f	3.0	7.2	188		19.0±0.42f	3.2
SOIL+GM	0.061		11.3±0.05g	5.9	20.8	328		11.1±0.42g	6.2
SOIL+SMS	0.027		25.3±0.71d	7.4	$2.5x10^4$	$9.1x10^5$		25.3±0.71d	7.8
LSD ($p<0.05$)			1.26					1.31	
1-month soil					Diazinon				
SOIL	0.068		10.1±0.64d	11.6	3.9	47.7		9.2±0.78e	12.0
SOIL+SS	0.094	16	23.4±0.35a	10.6	$4.7x10^4$	$5.0x10^5$	16	23.4±0.35a	13.2
SOIL+GM	0.117		5.9±0.21e	5.6	5.7	42.0		5.5±0.07f	4.8
SOIL+SMS	0.236	16	18.9±0.21b	9.6	1.2	2.3	16	17.8±0.28c*	3.8
12-month soil									
SOIL	0.034		20.2±1.84b	14.5	$6.6x10^4$	$1.9x10^6$		20.2±1.84b	15.7
SOIL+SS	0.049		14.0±0.49c	6.8	7.6	141		13.4±0.64d	7.1
SOIL+GM	0.076		9.1±0.14d	10.2	1.6	12.8		7.0±0.85f*	8.5
SOIL+SMS	0.162	20	24.3±0.00a	3.7	2.5	11.3	20	23.5±0.14a	3.0
LSD ($p<0.05$)			1.62					1.88	
1-month soil					Myclobutanil				
SOIL	0.017	22	62.0±1.20f	11.7	0.81	19.9	22	48.9±2.62e*	6.6
SOIL+SS	0.009	22	96.0±5.23c	6.9	0.63	30.7	22	83.3±2.05c*	4.8
SOIL+GM	0.016	28	70.7±2.33e	13.2	0.53	8.8	28	51.5±1.48e*	6.5
SOIL+SMS	0.007	22	125±9.76b	3.2	0.85	82.1	22	126±13.6b	2.3
12-month soil									
SOIL	0.010	16	84.3±5.16d	6.9	0.61	23.6	16	65.6±1.56d*	2.4
SOIL+SS	0.010	16	88.1±7.21d	3.3	$1.9x10^3$	$2.0x10^5$	16	88.1±7.21c	3.5
SOIL+GM	0.016	8	51.9±0.71g	3.3	18.0	$1.1x10^3$	8	51.2±0.21e	3.4
SOIL+SMS	0.006	16	137±23.5a	6.6	716	$1.2x10^5$	16	137±23.5a	6.9
LSD ($p<0.05$)			7.52					6.24	

[a] Average DT_{50} values (lag phase included) ± standard deviation.

The same letter in DT_{50} values within a column indicates that they are not significantly different and an asterisk in DT_{50} values in a file indicates that they are significantly different according to LSD between soil groups and kinetic models.

Figure
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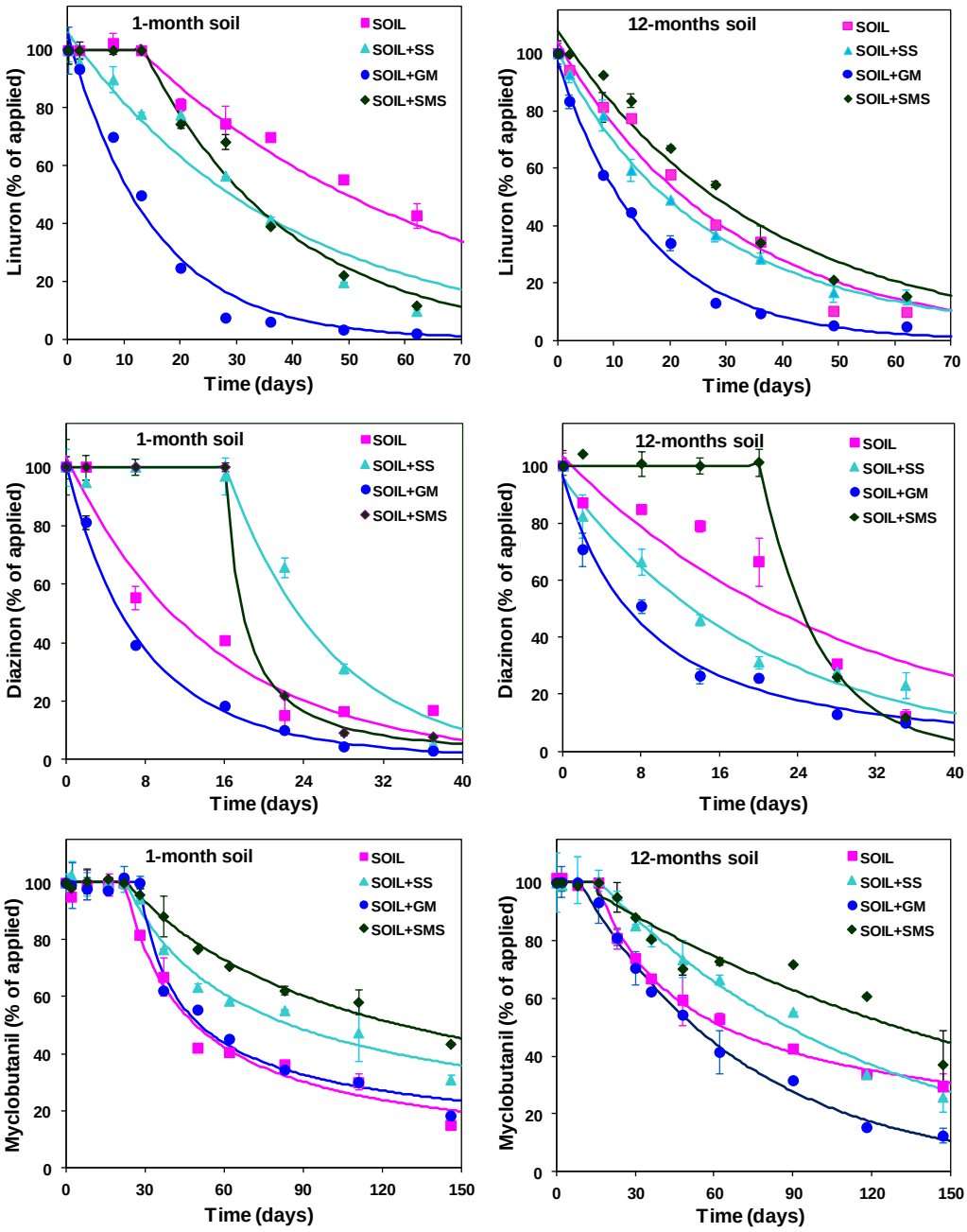

Fig. 1. Dissipation kinetics of linuron, diazinon and myclobutanil in unamended soil and in soil amended with sewage sludge (SS), grape marc (GM) and spent mushroom substrate (SMS) incubated for one month and 12 months. Bars indicate the standard deviation of the mean.

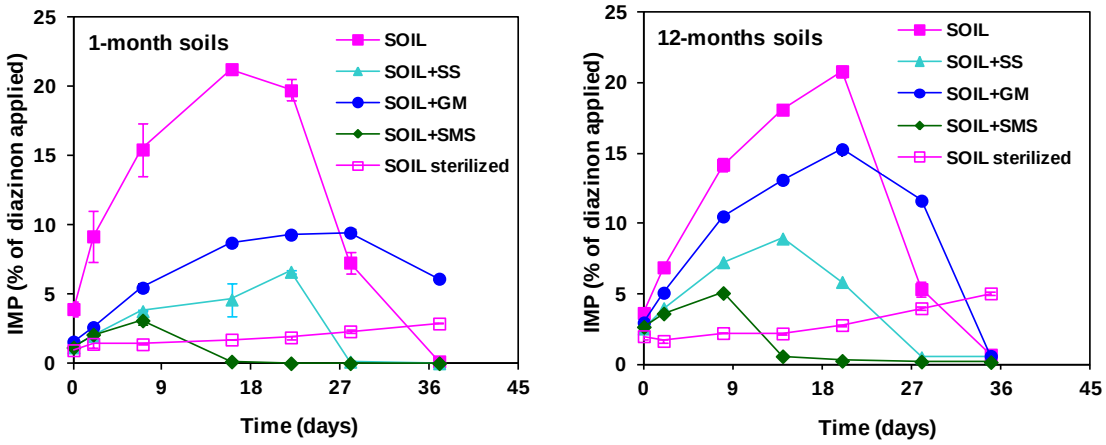

Fig. 2. Diazinon metabolite (IMP) formation over time in unamended soil and in soil amended with sewage sludge (SS), grape marc (GM) and spent mushroom substrate (SMS) incubated for one month and 12 months. Bars indicate the standard deviation of the mean.

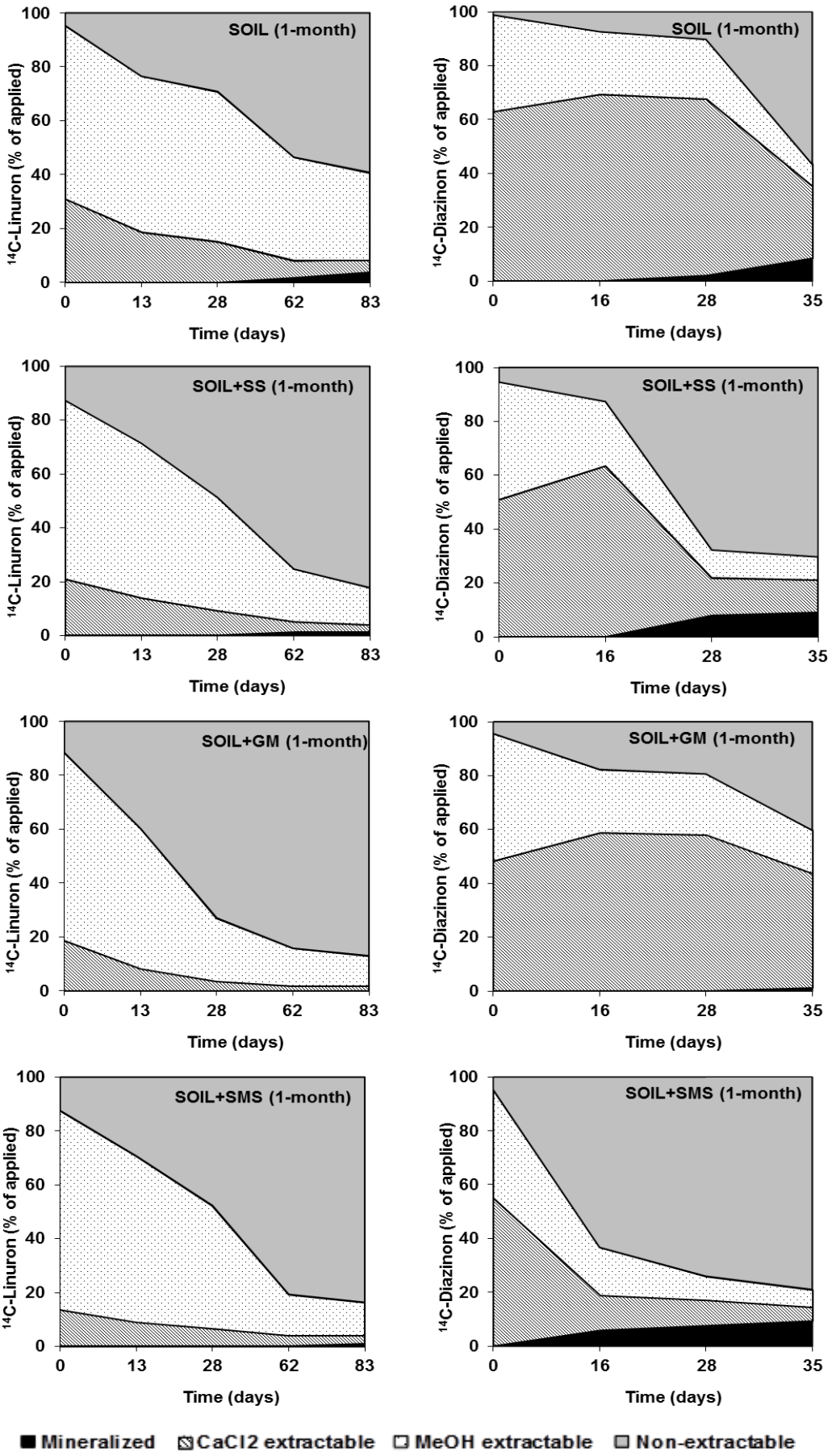

Fig. 3. Mass balance of mineralized, $CaCl_2$-extracted, methanol-extracted, and non-extracted ^{14}C (expressed as percentage of applied ^{14}C) for the dissipation studies of ^{14}C- linuron (left side) and ^{14}C-diazinon (right side) in unamended and amended soils incubated for one month

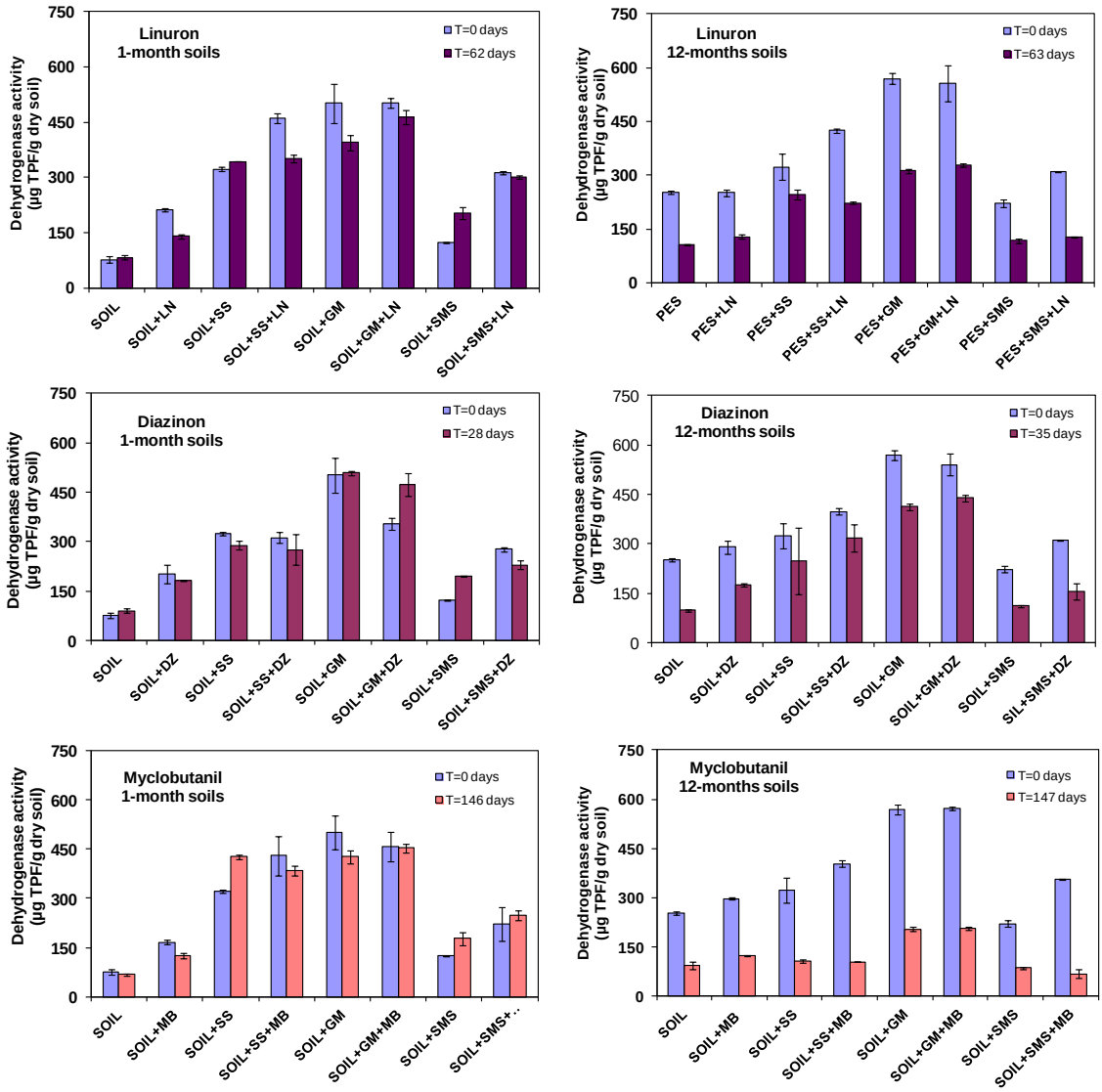

Fig. 4. Soil dehydrogenase activity for unamended and amended soil with sewage sludge (SS), grape marc (GM) and spent mushroom substrate (SMS), incubated for one month and 12 months, untreated and treated with pesticides linuron (LN), diazinon (DZ) and myclobutanil (MB) at the beginning and at the end of the incubation period. Bars indicate the standard deviation of the mean.