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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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1 **ABSTRACT**

2 Dissipation kinetics of pesticides belonging to three chemical groups (linuron, diazinon
3 and myclobutanil) was studied in an unamended agricultural soil and in this soil
4 amended with three organic residues: sewage sludge (SS), grape marc (GM) and spent
5 mushroom substrate (SMS). The soils were incubated with the residues outdoors for one
6 and 12 months. Mineralized, extracted and non-extractable fractions were also studied
7 for ¹⁴C-linuron and ¹⁴C-diazinon. The dissipation kinetics was fitted to single first-order
8 or first-order multicompartement models. The dissipation rate (k) decreased in the order
9 diazinon > linuron > myclobutanil, and DT₅₀ values decreased for linuron (1.6-4.8
10 times) or increased for myclobutanil (1.7-2.6 times) and diazinon (1.8-2.3 times) in the
11 amended soils relative to the unamended soil. The lowest DT₅₀ values for the three
12 pesticides were recorded in GM-amended soil, and the highest values in SMS-amended
13 soil. After 12 months of soil incubation, DT₅₀ values decreased in both the unamended
14 and amended soils for linuron, but increased for the unamended and SMS-amended soil
15 for diazinon and myclobutanil. A certain relationship was observed between the
16 sorption of pesticides by the soils and DT₅₀ values, although was significant only for
17 myclobutanil (p<0.05). Dissipation mechanism recorded the lowest mineralization of
18 ¹⁴C-pesticides in the GM-soil despite the highest dissipation rate in this soil. The
19 extracted ¹⁴C-residues decreased with incubation time, with increased formation of non-
20 extractable residues, higher in amended soils relative to the unamended soil. Soil
21 dehydrogenase activity was, in general, stimulated by the addition of the organic
22 amendments and pesticides to the soil after one month and 12 months of incubation.
23 The results obtained revealed that the simultaneous use of amendments and pesticides in
24 soils requires a previous study in order to check the environmental specific persistence
25 of these compounds and their effectiveness in amended soils.

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

28

29 **1. Introduction**

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

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

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

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

58 Dissipation of pesticides in amended soils can be decreased by the enhanced
59 sorption of these compounds by the OM of the amendments (Grenni et al., 2009; Marín-
60 Benito et al., 2012a,b; Rodríguez-Cruz et al., 2012b) although an apparent increased
61 dissipation may be also observed if irreversible sorption occurs with formation of bound
62 residues (Alexander, 2000). Furthermore dissipation can be affected if soil microbial
63 activity is stimulated by the addition of organic amendments and pesticide
64 biodegradation is enhanced (Moorman et al., 2001; Kadian et al., 2008). To date, a few
65 studies have studied the influence of selected residues on the degradation and
66 persistence of some pesticides in soils amended (Sánchez et al., 2004; Kadian et al.,
67 2008; Fernández-Bayo et al., 2009; Marín Benito et al., 2012b), but only in some of
68 them the dissipation mechanism has been evaluated.

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

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76 the adsorption (Rodríguez-Cruz et al., 2012a) and the mobility (Marín-Benito et al.,
77 2013) of linuron, diazinon and myclobutanil, in one-month and 12-month incubated soil
78 amended with SS, GM and SMS. The selected pesticides represent groups of
79 compounds with different chemical structures and widely used in agriculture. They are
80 applied in large amounts to a very wide range of crops to control annual grass and
81 broad-leaved weeds, insects and mites or fungal diseases (Tomlin, 2000). However, no
82 dissipation studies have been conducted in these amended soils, and further research is
83 required to assessment the risk of persistence of these compounds over time and their
84 possible contribution to soil and/or water pollution.

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

93 94 **2. Materials and methods**

95 **2.1. Chemicals**

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

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

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

117 **2.2. Organic residues**

118 Sewage sludge (SS) from a domestic waste treatment plant and stabilized by
119 anaerobic digestion was supplied by Aqualia SA (Salamanca, Spain). Grape marc
120 (GM), which is comprised of grape stalks, seeds and skins left after the crushing,
121 draining and pressing stages in wine production, was supplied by San Gabriel winery
122 (Aranda de Duero, Spain). Spent mushroom substrate (SMS) from *Agaricus bisporus*
123 (75%) and *Pleurotus sp.* (25%) cultivation is a pasteurized mixture of cereal straw and
124 poultry litter, ammonium nitrate, urea, and minerals (gypsum and/or calcium carbonate),
125 which was further composted for several weeks under aerobic conditions to obtain a

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

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

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140 **2.3. Unamended and amended soil**

141 The soil used in all experiments was a Typic Xerorthent sandy loam soil (Soil
142 Survey Staff, 2006) taken from the surface horizon (0-30 cm) of a vineyard farm in the
143 Castilla-Leon region (NW-Spain) located in Pesquera de Duero (41° 38' 34''N latitude
144 and 4° 9' 27'' W longitude). The soil was sieved (< 2 mm) and dried to determine their
145 characteristics using standard analytical methods (MAPA, 1986). The soil pH
146 determined in a soil/water suspension (1/2.5 w/v) was 7.9. The particle size distribution
147 determined using the pipette method was 76.9%, 8.2% and 14.9% sand, silt and clay,
148 respectively. The OC content determined by oxidation (Walkley-Black method) was
149 0.47%. The DOC was 0.04 mg g⁻¹, it was determined in soil extracts (1/2 w/v ratio) in
150 Milli-Q ultrapure water after soil shaking (24 h at 20°C), centrifugation (20 min at

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151 10000 rpm), and filtering (Minisart NY 25 filter 0.45 μ m, Sartorius Stedim Biotech,
152 Germany) using an organic carbon analyzer as previously indicated for organic residues.
153 Inorganic carbon content determined as CaCO₃ with a Bernard calcimeter was 2.25%.

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

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

166 Samples of unamended soil and soil amended with different residues were taken
167 after one month (one-month incubated soils) and 12 months (12-month incubated soils)
168 of incubation outdoors. They were sieved (<2 mm) and dried and the OC and DOC
169 contents and pH values were determined as previously indicated. In addition, alkali
170 soluble and acid insoluble carbon (humic acid, HA) and alkali and acid soluble carbon
171 (fulvic acid, FA) were also determined in unamended and amended soils. Soil extracts
172 were obtained following the traditional method of HA and FA extraction using 5 g of
173 soil and an initial solution of Na₄PO₇ and Na₂SO₄ in H₂SO₄ (25 mL) (Gallardo and
174 Bacas, 1973). The determination of C in solution was carried out as previously
175 described for the DOC. Results obtained are included in Table 2.

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

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180 **2.4. Dissipation studies**

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

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

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

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200 **2.5. Extraction and determination of pesticides**

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

216 Linuron, diazinon, and myclobutanil were quantified by HPLC with diode array
217 (DAD) and mass spectrometer (MS) detectors (Waters Associates, Milford, MA), using
218 a Waters Symmetry C18 column (75 × 4.6 mm i.d., 3.5 µm) at ambient temperature.
219 The mobile phase was 72:28 (v/v) methanol/ammonium formate 5 mM for linuron,
220 80:20 (v/v) methanol/water for diazinon, and 75:25 methanol/water (0.1 % formic acid)
221 for myclobutanil. The flow rate of the mobile phase was 0.3 mL min⁻¹ and the sample
222 injection volume was 10 µL. The retention time was 6.8, 7.2, and 6.3 min for linuron,
223 diazinon, and myclobutanil. Quantitative analysis was performed using the peak area of
224 each compound obtained from the total ion chromatogram (TIC) in SIM mode. The
225 molecular ions (m/z) corresponding to each pesticide in the positive ionization mode

226 [M]⁺ were 250.1, 305.2, and 289.1 for linuron, diazinon, and myclobutanil, respectively.
227 Calibration was performed from 0.05 to 2.5 µg mL⁻¹ and the limit of detection (LOD)
228 and limit of quantification were >0.01 and >0.03 µg mL⁻¹, respectively, for all of the
229 pesticides.

230 Monitoring also involved positive molecular ions (m/z) 220.1, 236.1, 206.1 and
231 162.0 for linuron metabolites (N-(3,4-dichlorophenyl)-N'-methylurea, N-(3,4-
232 dichlorophenyl)-N'-methoxyurea, N-(3,4-dichlorophenyl)urea and 3,4-dichloroaniline),
233 153.2 for diazinon metabolite (2-isopropyl-6-methyl-4-pyrimidinol), and 128.1 for
234 myclobutanil metabolite (1-H-1,2,4-triazol-1-ylacetic acid). The myclobutanil
235 metabolite was only qualitatively monitored.

237 **2.6. Mineralization and mass balance of ¹⁴C-linuron and ¹⁴C-diazinon**

238 For linuron and diazinon, simultaneous incubations were carried out with ¹⁴C-
239 labeled pesticides to study the dissipation mechanism (mineralization kinetics and the
240 formation of non-extractable residues over time). Aqueous solutions of unlabeled
241 pesticide of an appropriate concentration were labeled with ¹⁴C-pesticides, and a volume
242 of 10 mL of these solutions was added to 300 g of fresh weight of unamended or
243 amended soils to give a concentration of 2 mg kg⁻¹ dry soil and an approximately
244 activity of 100 Bq g⁻¹. In these soil samples, a ¹⁴CO₂ trap, consisting of a scintillation
245 vial containing 1 M NaOH (1 ml), was attached to the lid via a stainless steel clip as
246 described by Reid et al. (2002).

247 The extraction of the ¹⁴C-pesticides from the soil was carried out in two
248 sequential steps: Initially the ¹⁴C-pesticide was extracted with 10 mL of a 0.01 M CaCl₂
249 Milli-Q ultrapure water solution for 24 h, and after a second extraction with 10 mL of
250 the organic solvent methanol was carried out for 24 h.

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251 The quantitative determination of ^{14}C -linuron and ^{14}C -diazinon after extraction
252 was performed by liquid scintillation using a Beckman LS6500 liquid scintillation
253 counter (Beckman Instruments Inc., Fullerton, CA, USA). The radioactivity of the
254 solution was measured in disintegrations per minute (dpm), being determined in
255 duplicate in 1 mL of aqueous or methanol extracts to which 4 mL of scintillation
256 cocktail was added (Ecoscint TMA, National Diagnostics, Atlanta, GA, USA). Residues
257 of ^{14}C -pesticides remaining in the soil after extraction were determined by the
258 combustion of triplicate 1 g dried soil samples, using a Biological Oxidizer (R.J. Harvey
259 OX-500 Instrument Corp., NJ) under O_2 excess at $900\text{ }^\circ\text{C}$. The $^{14}\text{CO}_2$ generated was
260 trapped in a mixture of ethanolamine (1 mL) and scintillation cocktail (Oxisolve C-400,
261 Zinsser Analytic, Berkshire, UK; 15 mL) and determined as indicated above.

262 $^{14}\text{CO}_2$ from mineralized ^{14}C -pesticides in the scintillation vial containing 1 M
263 NaOH (1 mL) was determined at the different sampling times by mixing with 4 mL of
264 scintillation cocktail and determined as previously indicated.

266 **2.7. Soil dehydrogenase activity**

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

272 **2.8. Data analysis**

273 The dissipation kinetics for the pesticide was fitted to a single first-order (SFO)
274 kinetic model ($C = C_0 e^{-kt}$) or first order multicompartiment (FOMC) model ($C = C_0 / ((t$
275 $/ \beta) + 1)^\alpha$), known also as the Gustafson and Holden model. C is the pesticide

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276 concentration at time t , C_0 is the initial pesticide concentration, k (day^{-1}) is the
277 dissipation rate, α is a shape parameter determined by the coefficient of variation of k
278 values and β is a location parameter. For the selection of the kinetic model that best
279 describes the dissipation results, FOCUS work group guidance recommendations were
280 followed (FOCUS, 2006) The coefficient of determination (r^2) and the chi-square (χ^2)
281 test were calculated as indicators of the goodness of fit. The χ^2 test considers the
282 deviations between observed and calculated values relative to the uncertainty of the
283 measurements for a specific fit, and was used to compare the goodness of fit of the two
284 models tested. The error value at which the χ^2 test is fulfilled at a given degree of
285 freedom should be below 15 % (at 5 % significance level). Values for the time to 50%
286 dissipation, or DT_{50} values, were used to characterize the decay curves and compare
287 variations in dissipation rates. The parameters of the kinetic models were estimated
288 using the Excel Solver add-in package (FOCUS, 2006).

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289 Analysis of variance (ANOVA) was used to evaluate the effects of the different
290 treatments on the dissipation of pesticides. Standard deviation (SD) was used to indicate
291 variability among replicates, and the least significant difference (LSD), at a confidence
292 level of 95 %, was determined to evaluate the effects of different soil treatments on
293 DT_{50} values. Statgraphics Plus version 5.1 statistical software (Statgraphics Plus Corp.,
294 Princeton, NJ, USA) was used.

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296 **3. Results and discussion**

297 **3.1. Dissipation kinetics of pesticides in the unamended and amended soil**

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

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

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

317 The dissipation kinetics of diazinon also fitted the SFO model in the unamended
318 soil and the SS-amended soil after one month and 12 months of incubation. However,
319 diazinon dissipation fitted the FOMC model better in the GM- and SMS-amended soil
320 for both incubation times. Diazinon dissipation initially recorded a lag phase of 16 days
321 (Soil+SS) or 16-20 days (Soil+SMS), and it was followed by a rapid pesticide
322 dissipation phase. Different models with or without lag phase have also been reported in
323 the literature to fit the dissipation curves of diazinon in unamended soils and soils
324 treated with municipal waste water and surfactant solutions (Hernández-Soriano et al.,
325 2009; Cycon et al., 2010a).

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

332 The dissipation rate (k) decreased in the order diazinon > linuron > myclobutanil
333 in the unamended soil. DT₅₀ values in days for the dissipation of the pesticides studied
334 were 49.8 and 21.2 (linuron), 10.1 and 20.2 (diazinon) and 48.9 and 65.6 (myclobutanil)
335 in the unamended soil after one month and 12 months of incubation. Changes in the
336 DT₅₀ values of pesticides were significant (Table 3) after unamended soil ageing,
337 although changes in the soil characteristics (OM, sorption parameters, etc.) were not
338 significant. These results are in agreement with the wide range of DT₅₀ values found in
339 the literature (Table 1) for the dissipation of these pesticides in different unamended
340 soils (FOOTPRINT, 2011).

341 After soil amendment, the dissipation of diazinon and myclobutanil was slower
342 in the SS- and SMS-amended soil than in the unamended one. Note should be taken of
343 the existence of a lag phase before a rapid dissipation phase in these soils. This effect
344 was expected, given the increase in the sorption of both pesticides by amended soils
345 relative to the unamended one and the influence of sorption on the dissipation kinetics
346 of pesticides in soils due to a decrease in the bioavailability and biodegradation of
347 organic compounds sorbed by the soil (Alexander, 2000). DT₅₀ values increased 1.7 and
348 2.6 times for myclobutanil, and 2.3 and 1.8 times for diazinon in the SS- and SMS-
349 amended soil, respectively. However, the dissipation in GM-amended soil was similar

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2 350 for myclobutanil or increased for diazinon (DT_{50} values decreased 1.8 times relative to
3 351 the unamended soil).

4 352 An opposite effect was observed for the dissipation of linuron; it was more rapid
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6 353 in the amended soils than in the unamended one, although the sorption of linuron by the
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8 354 amended soils increased (Table 1). DT_{50} values decreased between 1.6 and 4.8 times in
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10 355 the amended soils. According to these results, it may be assumed that different
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12 356 mechanisms are involved in the dissipation of pesticides in amended soils.
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16 357 Regarding the three pesticides, the lowest DT_{50} values were generally obtained
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18 358 in the GM-amended soil, with the highest in the SMS-amended soil (Table 3). The GM-
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20 359 amended soil has the highest DOC content (Table 2), and the pesticides could be sorbed
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22 360 by the DOC in soil solution, to a greater or lesser extent increasing their availability for
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24 361 degradation (Barriuso et al., 2011). The highest DT_{50} values of linuron and
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26 362 myclobutanil in the SMS-amended soil could be explained by the high sorption of these
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28 363 pesticides by this soil (Table 1) with more stabilized OM, taking into account that the
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30 364 HA/FA ratio was the highest among the amended soils (Table 2).
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35 365 DT_{50} values decreased for linuron in the SS- and SMS-amended soil after 12
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37 366 months of incubation, and increased for diazinon and myclobutanil in the SMS-
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39 367 amended soil with regard to the corresponding soil after one month of incubation (Table
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41 368 3). No significant differences were found in the DT_{50} values for pesticides in the GM-
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43 369 amended soil. Moreover, for diazinon, dissipation was more rapid in the SS-amended
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45 370 soil due to the suppression of the lag phase recorded in the homologous one-month
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47 371 incubated soil. The lag phase was also shorter for the dissipation of myclobutanil in the
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49 372 12-month incubated soils than in the one-month incubated soil. This indicated that the
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51 373 adaptation time needed for the microbial community to degrade myclobutanil and
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2 374 diazinon in some amended soils was shorter in the 12-month incubated soils, possibly
3 375 due to the decrease in OC and DOC in these soils (Table 2).

4 376 Dissipation could be related to the sorption behaviour of the studied pesticides
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6 377 by soils, in keeping with the decrease or increase in their sorption by the amended soils
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8 378 after one-month and 12-month ageing, as discussed by the authors in a previous work
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10 379 (Rodriguez-Cruz et al., 2012a). However, the sorption coefficients of linuron and
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12 380 diazinon by soils (Table 1) were not significantly correlated with the DT₅₀ values when
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14 381 one-month and 12-month incubated soils were considered in the analysis. A positive
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16 382 and significant correlation ($r=0.79$, $p<0.05$) between the sorption coefficients and DT₅₀
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18 383 values was found solely for myclobutanil when one-month and 12-month incubated
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20 384 soils were considered together.

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22 385 The influence of sorption on the degradation kinetics of some fungicides in
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24 386 different SMS-amended soils has been observed in previous studies (Marín-Benito et
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26 387 al., 2012b). Regarding the pesticides studied here, Rodríguez-Cruz et al. (2001) have
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28 388 reported a decrease in the degradation rate of linuron in soils with a liquid humic
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30 389 amendment and peat due to herbicide adsorption by amended soils. However, the
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32 390 degradation of linuron in city refuse compost-amended soil increased due to an increase
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34 391 in the microbial activity after soil amendment. The linuron half-life values indicated a
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36 392 slower degradation rate in pine- and oak-amended soils than in unamended ones (Grenni
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38 393 et al., 2009). A decrease in the dissipation rate of diazinon in soil amended with sewage
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40 394 sludge was observed and attributed to a reduction in its availability and, therefore, a
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42 395 greater persistence compared to the untreated soil (Sánchez et al., 2004). The
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44 396 degradation of diazinon in neem cake-amended soils was prolonged compared to soils
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46 397 without amendment, increasing the persistence of the insecticide (Akhtar et al., 1998).

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

400 Results of the dissipation of pesticides in sterilized unamended soil indicated
401 that the soil microbial community played an active role in this process, as no actual
402 dissipation was observed, or it was much slower than for non-sterilized soil (data not
403 shown). However, the dissipation data for linuron in the 12-month incubated sterilized
404 soil (76% remained after 62 days) and diazinon in the one-month incubated sterilized
405 soil (81% remained after 37 days) suggest the influence of other abiotic factors, such as
406 chemical hydrolysis, as previously reported (Rodríguez-Cruz et al., 2001; Sarmah et al.,
407 2009).

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

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

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

428 429 **3.2. Mass balance of ¹⁴C-linuron and ¹⁴C-diazinon in the unamended and amended** 430 **soil**

431 The total ¹⁴C balance corresponding to mineralized, extracted (as parent or
432 metabolites), and non-extractable (bound residues) ¹⁴C-linuron and ¹⁴C-diazinon was
433 determined to explain dissipation mechanism in the unamended and amended soil
434 incubated for one month (Figure 3) and in unamended and amended soil incubated for
435 12 months (data not-shown). The total mass balance (expressed as a percentage of the
436 ¹⁴C initially applied) was, in general, >84% for ¹⁴C-linuron (79-95% range) and >80%
437 for ¹⁴C-diazinon (70-104% range).

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

442 Linuron mineralization was low in all the soils and increased slowly over the
443 incubation period. The amounts of ¹⁴C-linuron mineralized to ¹⁴CO₂ after 83 days were
444 2.45%, 1.16%, 0.38% and 0.67%, in unamended soil, soil+SS, soil+GM and soil+SMS,
445 respectively, after one month of incubation, and 2.14%, 1.15%, 0.92% and 1.62%, in
446 the respective unamended and amended soils after 12 months of incubation. The lower
447 values were recorded in the amended soils, even though the dissipation rates were

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

453 Mineralization was initially very slow for diazinon in the unamended and
454 amended soils, although a higher increase in $^{14}\text{CO}_2$ evolution was observed in the SS-
455 and SMS-amended soil over time. This low initial mineralization must correspond to the
456 previous adaptation period of the soil microbial community, according to the lag phase
457 observed in some of the amended soils (Figure 1), and biodegradation could then occur
458 more quickly. The percentages mineralized after 35 days were 8.51%, 6.21%, 1.09%
459 and 6.74%, in unamended soil, and SS-, GM- and SMS-soil, respectively, after one
460 month of incubation. These amounts were 5.45%, 7.98%, 4.22% and 6.68% in 12-
461 month incubated soils. ^{14}C -Diazinon mineralization in all the soils was higher than that
462 of linuron, possibly because the ^{14}C -label of diazinon is located in the side methyl
463 group, compared to the ^{14}C -label of linuron on the ring, and may therefore allow a
464 greater production of $^{14}\text{CO}_2$. The lower mineralization was recorded in the unamended
465 soil and GM-amended soil despite the highest dissipation rates found in these soils. This
466 is consistent with the formation of a high amount of metabolite IMP in these soils
467 (Figure 2), which must be degraded to $^{14}\text{CO}_2$ more slowly, as mineralization kinetics
468 indicates. In relation to this, a report drafted by the National Registration Authority of
469 Australia (NRA, 2002) has indicated that IMP is slowly degraded and mineralized to
470 $^{14}\text{CO}_2$ in soils.

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471 The extracted amounts in CaCl₂ and methanol decreased for both pesticides as
472 incubation time increased. In general, the decrease was more rapid in the amended soils
473 with respect to the unamended one after both soil incubation periods (Figures 3).

474 Soil extraction with water solutions (0.01M CaCl₂) provides an estimate of the
475 availability of pesticide residues to be degraded, and depends mainly on pesticide
476 sorption (Mamy et al., 2005). For linuron the ¹⁴C amounts in water extracts were
477 initially higher in the unamended soil (up to 27%) than in the amended ones (up to
478 18%), although they were similar in both one-month and 12-month incubated soils
479 (Figure 3). These amounts were time dependent and decreased quickly after 83 days in
480 the unamended (< 3%) and amended soils (<2%) incubated for one and 12 months.
481 These results are consistent with the linuron sorption coefficients (Table 1), which were
482 higher for the amended soils.

483 However, the amounts of ¹⁴C-linuron extractable in methanol were initially very
484 similar in the unamended and amended soils (55-68%), but the decrease was higher in
485 the amended soils with respect to the unamended soil as indicated for non-labeled
486 pesticides (Figure 3). The extracted ¹⁴C amounts were 19.4% (unamended soil) and
487 11.7%, 10.2%, and 9.48% (SS-, GM-, and SMS-amended soil) of the ¹⁴C-linuron
488 initially added after 83 days in one-month incubated soils, and 15.6% (unamended soil)
489 and 10.3%, 9.50%, and 9.98% (SS-, GM-, and SMS-amended soil) after 83 days in 12-
490 month incubated soils. The higher amount of extracted pesticide in the unamended soil
491 with regard to the amended soils was consistent with the slowest dissipation rate in this
492 soil.

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

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496 incubation period in the unamended and amended soils in both one-month and 12-
497 month incubated soils (Figure 3). These amounts decreased rapidly in the SS-amended
498 and SMS-amended soils up to <10%, but changes in the extracted amounts in the
499 unamended and GM-amended soils were smaller, and they were still high after 35 days,
500 26-37% in one-month incubated soils, and 22-25% in 12-month incubated soils (Figure
501 3).

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

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

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

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

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543 **3.3. Soil dehydrogenase activity**

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

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

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

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

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581 **4. Conclusions**

582 This study revealed a different effect of organic residues (SS, GM and SMS)
583 applied as amendments on the dissipation of pesticides in an amended agricultural soil.
584 Compared to unamended soil the DT₅₀ values of pesticides decreased for linuron in all
585 amended soils, and increased for diazinon and myclobutanil in the SS- and SMS-
586 amended soil. The dissipation was higher for all the pesticides in the GM-amended
587 soils, and lower in the SMS-amended soils. The highest DOC content of GM-soil could
588 enhance the sorption by the DOC in soil solution increasing their availability for
589 degradation while the more stabilized OM of SMS-soil could increase the sorption of
590 pesticides. The effect of soil ageing on dissipation was consistent with the changes in
591 the sorption of pesticides by soils after incubation. Different dissipation mechanisms
592 were revealed for ¹⁴C-linuron and ¹⁴C-diazinon. Mineralized and extractable amounts
593 were higher for diazinon than for linuron. For both pesticides, the extractable amounts
594 decreased and the non-extractable amounts increased as incubation time increased,
595 which is consistent with the dissipation rates. The effect of soil ageing was seen mainly

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

603

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740 **Figure captions**

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4 742 **Fig. 1.** Dissipation kinetics of linuron, diazinon and myclobutanil in unamended soil
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7 743 and in soil amended with sewage sludge (SS), grape marc (GM) and spent mushroom
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9 744 substrate (SMS) incubated for one month and 12 months. Bars indicate the standard
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11 745 deviation of the mean.

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16 747 **Fig. 2.** Diazinon metabolite (IMP) formation over time in unamended soil and in soil
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19 748 amended with sewage sludge (SS), grape marc (GM) and spent mushroom substrate
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21 749 (SMS) incubated for one month and 12 months. Bars indicate the standard deviation of
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24 750 the mean.

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28 752 **Fig. 3.** Mass balance of mineralized, CaCl₂-extracted, methanol-extracted, and non-
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31 753 extracted ¹⁴C (expressed as percentage of applied ¹⁴C) for the dissipation studies of ¹⁴C-
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33 754 linuron (left side) and ¹⁴C-diazinon (right side) in unamended and amended soils
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36 755 incubated for one month

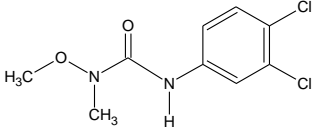
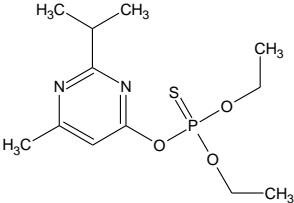
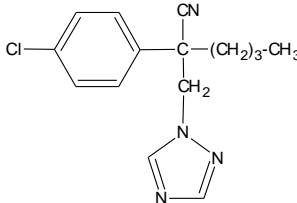
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41 757 **Fig. 4.** Soil dehydrogenase activity for unamended and soil amended with sewage
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43 758 sludge (SS), grape marc (GM) and spent mushroom substrate (SMS), incubated for one
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46 759 month and 12 months, untreated and treated with pesticides linuron (LN), diazinon (DZ)
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48 760 and myclobutanil (MB) at the beginning and at the end of the incubation period. Bars
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51 761 indicate the standard deviation of the mean.

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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)	La g (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 DT₅₀ values (lag phase included) ± standard deviation.

The same letter in DT₅₀ values within a column indicates that they are not significantly different and an asterisk in DT₅₀ values in a file indicates that they are significantly different according to LSD between soil groups and kinetic models.

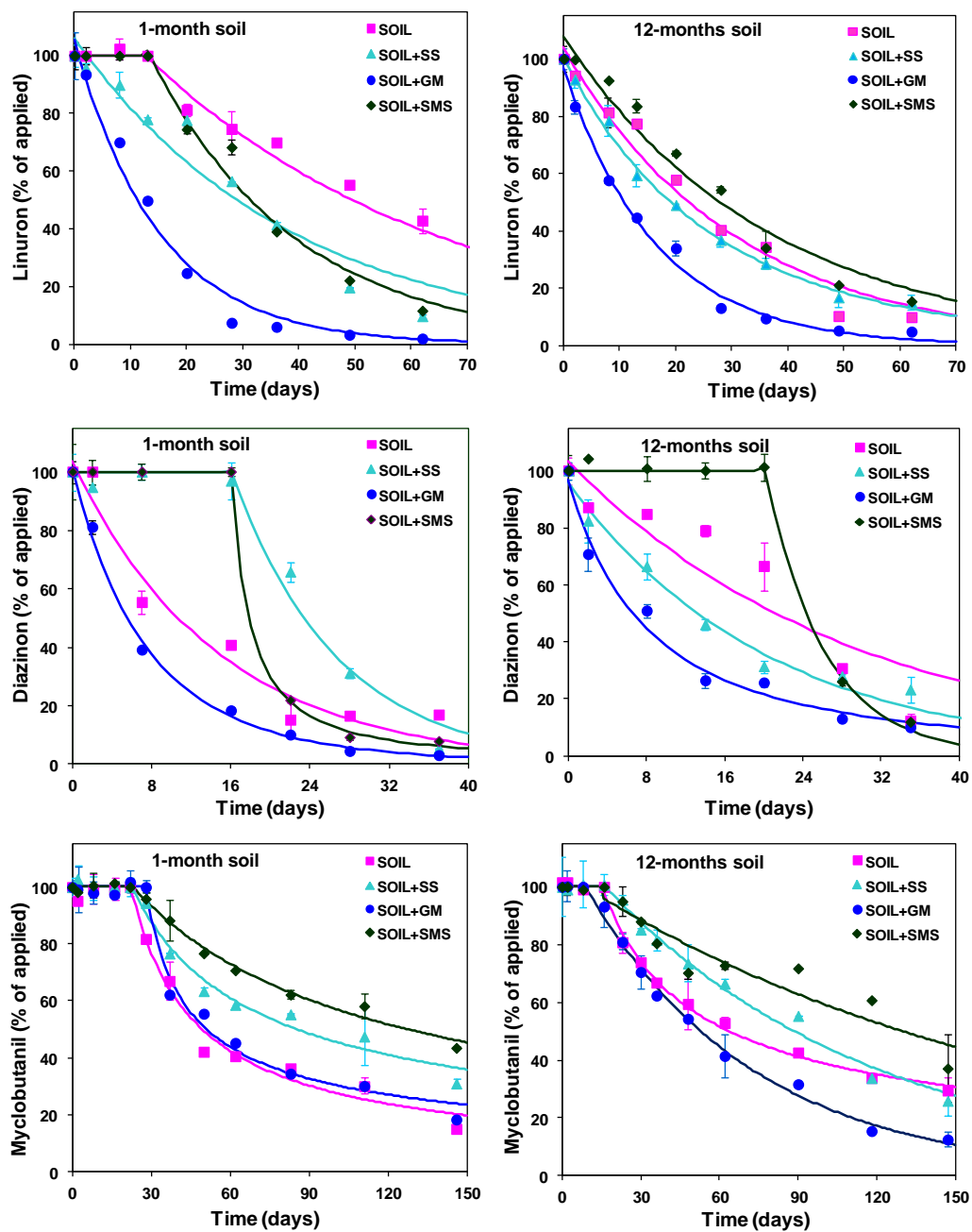


Fig. 1. Dissipation kinetics of linuron, diazinon and myclobutanil in unamended soil and in soil amended with sewage sludge (SS), grape marc (GM) and spent mushroom substrate (SMS) incubated for one month and 12 months. Bars indicate the standard deviation of the mean.

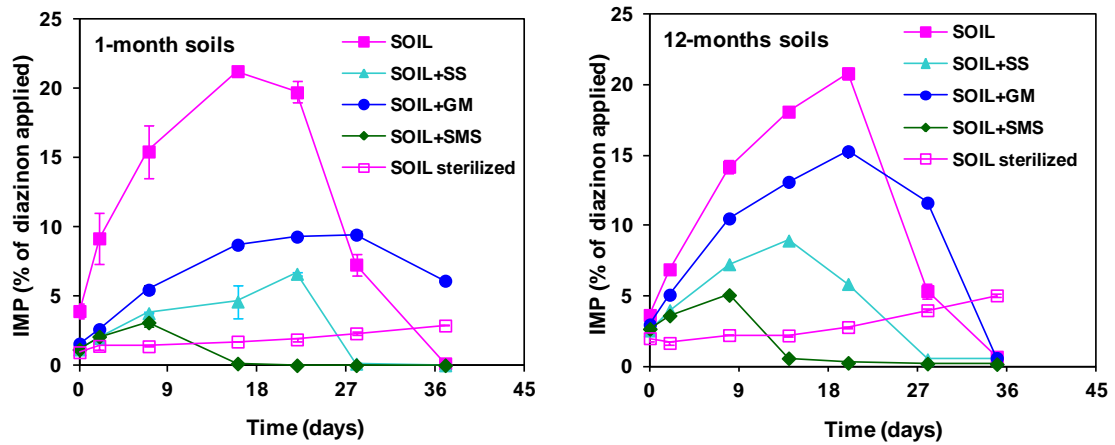


Fig. 2. Diazinon metabolite (IMP) formation over time in unamended soil and in soil amended with sewage sludge (SS), grape marc (GM) and spent mushroom substrate (SMS) incubated for one month and 12 months. Bars indicate the standard deviation of the mean.

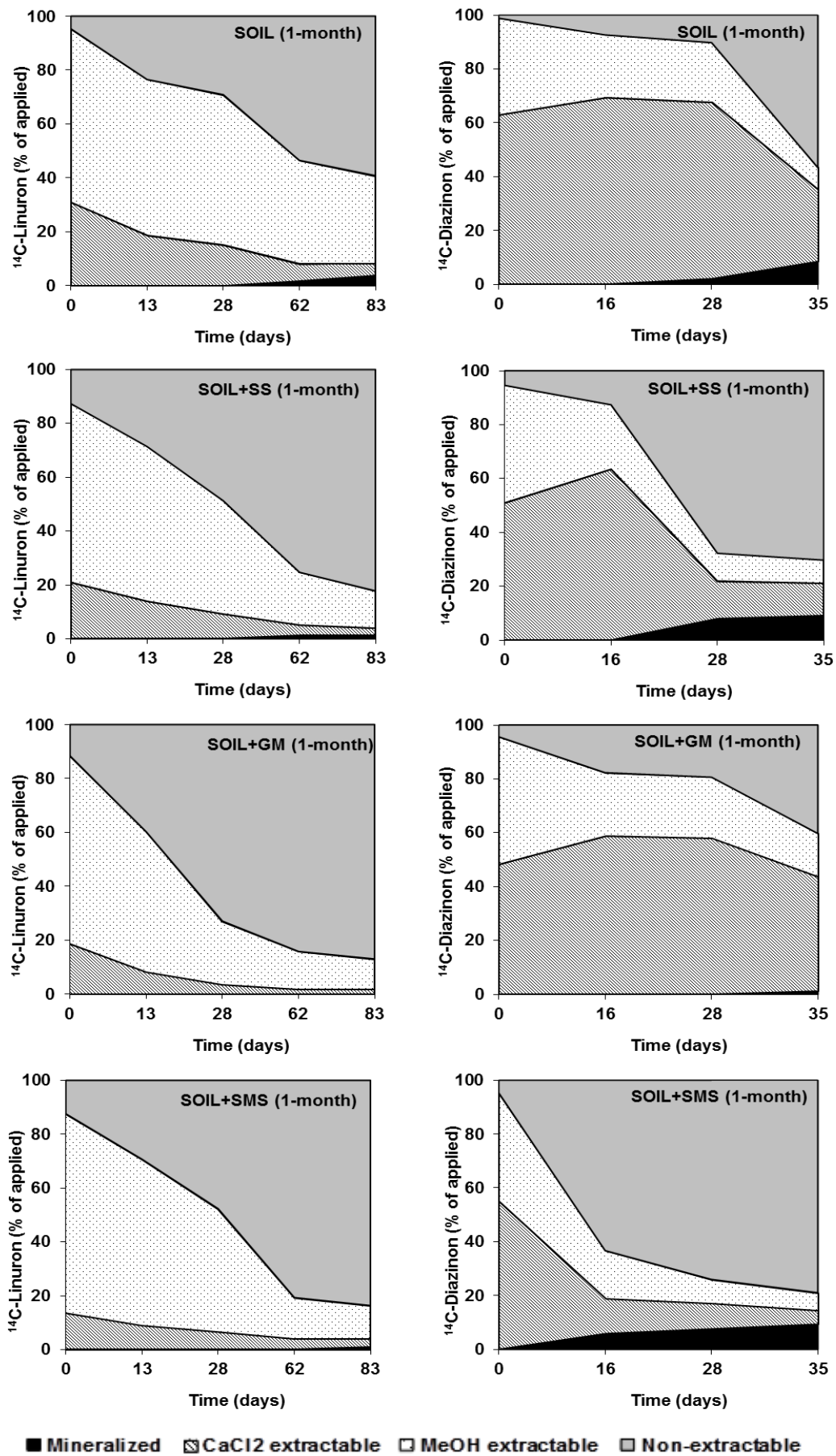


Fig. 3. Mass balance of mineralized, CaCl₂-extracted, methanol-extracted, and non-extracted ¹⁴C (expressed as percentage of applied ¹⁴C) for the dissipation studies of ¹⁴C- linuron (left side) and ¹⁴C-diazinon (right side) in unamended and amended soils incubated for one month

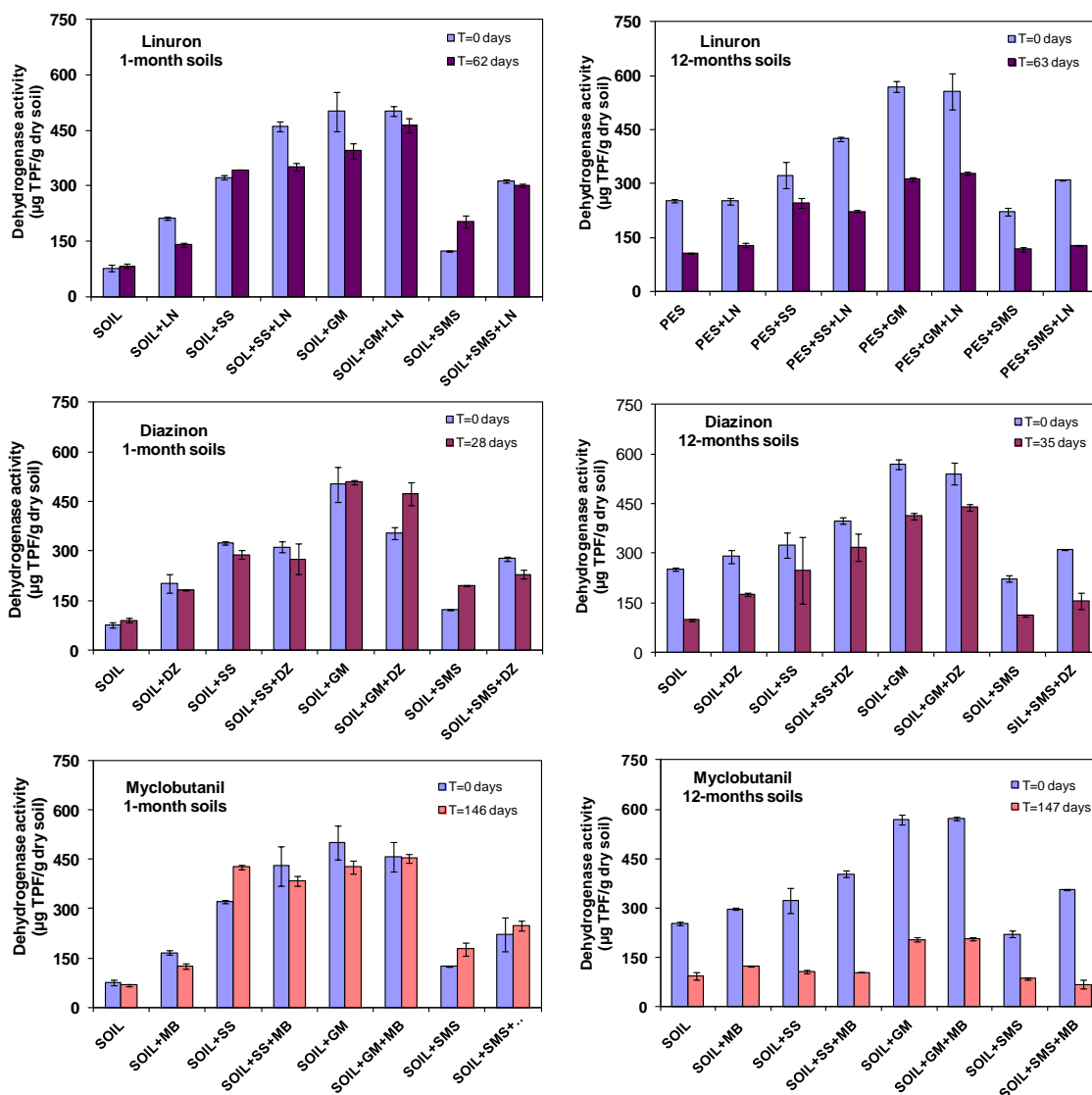


Fig. 4. Soil dehydrogenase activity for unamended and amended soil with sewage sludge (SS), grape marc (GM) and spent mushroom substrate (SMS), incubated for one month and 12 months, untreated and treated with pesticides linuron (LN), diazinon (DZ) and myclobutanil (MB) at the beginning and at the end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