



**HAL**  
open science

## Plants affect the dissipation and leaching of anilide pesticides in soil mesocosms: Insights from compound-specific isotope analysis (CSIA)

Paula Pérez-Rodríguez, Anne-Desirée Schmitt, Sophie Gangloff, Jérémy Masbou, Gwenael Imfeld

### ► To cite this version:

Paula Pérez-Rodríguez, Anne-Desirée Schmitt, Sophie Gangloff, Jérémy Masbou, Gwenael Imfeld. Plants affect the dissipation and leaching of anilide pesticides in soil mesocosms: Insights from compound-specific isotope analysis (CSIA). *Agriculture, Ecosystems & Environment*, 2021, 308, pp.107257. 10.1016/j.agee.2020.107257 . hal-03378994

**HAL Id: hal-03378994**

**<https://hal.science/hal-03378994>**

Submitted on 18 Oct 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Plants affect the dissipation and leaching of anilide pesticides in soil**  
2 **mesocosms: insights from compound-specific isotope analysis (CSIA)**

3  
4  
5 *Paula Pérez-Rodríguez<sup>a,b\*</sup>, Anne-Desirée Schmitt<sup>a</sup>, Sophie Gangloff<sup>a</sup>, Jérémy Masbou*  
6 *<sup>a</sup>, Gwenaël Imfeld<sup>a\*</sup>*

7  
8  
9  
10 <sup>a</sup> Université de Strasbourg, CNRS, ENGEES, LHyGeS UMR 7517, 1 rue Blessig,  
11 67000 Strasbourg, France

12 <sup>b</sup> Plant Biology and Soil Science Department, Soil Science and Agricultural Chemistry  
13 Area, Facultade de Ciencias de Ourense, As Lagoas s/n 32004, Ourense, Spain

14  
15  
16  
17 \*Corresponding author: paulaperezr@uvigo.es and imfeld@unistra.fr

18  
19  
20  
21  
22  
23  
24  
25  
26 Manuscript for Agriculture, Ecosystems & Environment - Journal

27 **Abstract**

28 The soil-plant system can act as a prevailing zone of pesticide dissipation in  
29 agroecosystems, which may influence pesticide leaching following rainfall events. Here  
30 we examined the contribution of leaching, dissipation and degradation of widely used  
31 anilide pesticides in planted and unplanted mesocosms with contrasting vineyard and  
32 forest soils. The mesocosms were spiked at 25 mg/kg with acetochlor, alachlor, *S*-  
33 metolachlor, butachlor and metalaxyl, and followed-up for 75 days. Successive rainfalls  
34 were applied on days 45, 60 and 75 to collect pesticide leachates. Bulk soil and  
35 rhizosphere samples were collected to evaluate pesticide dissipation. Up to 14% of  
36 initially spiked pesticides was exported by leaching. The first rainfall accounted for 69  
37 to 99% of the total pesticide leaching. Pesticide dissipation in soil mesocosms on day 75  
38 ranged from 58 to 99% and was larger in planted than in unplanted mesocosms.  
39 Compound-specific stable isotope analysis (CSIA) of the pesticides showed that  
40 biodegradation in both the vineyard and forest soil mesocosms occurred mainly in the  
41 soil solution that was leached following rainfall events rather than in the bulk soil.  
42 Changes in carbon isotope values ( $\Delta\delta^{13}\text{C}$  up to  $13.9 \pm 0.5\text{‰}$ ) of acetochlor, alachlor, *S*-  
43 metolachlor and butachlor in leachates were more pronounced in planted than in  
44 unplanted mesocosms, indicating predominant pesticide degradation in the planted  
45 mesocosms. Pesticide biodegradation was favoured in the soil solution of soil-plant  
46 systems, independently of the soil type. Leaching of the soil solution, pesticide ageing  
47 and biodegradation in the bulk soil and the rhizosphere were the main processes of  
48 pesticide dissipation. Overall, this study emphasizes the variability of leaching and  
49 degradation of anilide pesticides in agricultural soils, and proposes a framework using  
50 CSIA to examine the contribution of dissipation processes in soil-plant systems.

51

52 **Keywords:** Anilide pesticides; Biodegradation; Carbon isotope fractionation; Leaching;  
53 Mesocosms; Rhizosphere; Soil mesocosms.

54

## 55 **1. Introduction**

56 Anilide pesticides include widely used herbicides to control broad-leaf weeds  
57 and annual grasses in crops and fungicides commonly used in vineyards (EUROSTAT,  
58 2020). Anilide pesticides such as the herbicides acetochlor, alachlor, S-metolachlor,  
59 butachlor and the fungicide metalaxyl can be exported off-site from agricultural fields  
60 during rainfall events, representing a risk for ground and surface waters. Off-site export  
61 of pesticides from agricultural settings largely depends on their dissipation in the soil,  
62 including pesticide loss by leaching following a rainfall event, as well as plant uptake,  
63 and ageing and degradation in the soil. Although agricultural plots are generally  
64 vegetated, leaching and biodegradation of anilide pesticides in the soil-plant system are  
65 generally studied independently.

66 Interactions between plants, soil and microorganisms can enhance pesticide  
67 availability and dissipation (Chaudhry et al., 2005), making the soil-plant system an  
68 environmental hotspot for pollutant transformation (Kuzyakov and Blagodatskaya,  
69 2015). The soil-plant system includes the rhizosphere, which is characterized by diverse  
70 and intense microbial activity, respiration processes and nutrient uptake, mainly  
71 controlled by the water circulation (Bardgett and Van Der Putten, 2014; Hinsinger, et  
72 al., 2009; Hinsinger, et al., 2006; Mommer et al., 2016; Zhang et al., 2017). While  
73 organic contaminants are hardly adsorbed to organic matter and soil minerals, the root  
74 exudates from plants favour their desorption and availability, being directly accessed by  
75 macro- and microorganisms and plants (Huang et al., 2016). Plant rhizosphere can  
76 enhance pesticide dissipation by sustaining specific microbial communities capable of

77 pesticide transformation (Diez et al., 2017), resulting in shorter pesticide half-lives in  
78 the rhizosphere compared to the bulk soil (Hand et al., 2020). However, pesticide  
79 dissipation in the soil-plant system remains poorly understood as it depends mainly on  
80 the contribution of both degradative and non-degradative processes, which are  
81 themselves related to pesticide physicochemical properties and bioavailability  
82 (Chaudhry et al., 2005; Ren et al., 2018; Torabi et al., 2020).

83 Sorption, dilution and off-site transport can act simultaneously with degradation  
84 to dissipate pesticides in agricultural soil and making the dissipation difficult to  
85 disentangle using a mass balance approach relying on concentration analysis only.  
86 Compound-specific stable isotope analysis (CSIA) may provide a concentration-  
87 independent evaluation of pesticide degradation in the environment (Elsner and Imfeld,  
88 2016) by evaluating changes in the stable isotope ratios (e.g.,  $^{13}\text{C}/^{12}\text{C}$ , and  $^{15}\text{N}/^{14}\text{N}$ )  
89 within the molecule. Dilution processes such as transport or sorption, generally do not  
90 alter stable isotope ratios. However, pesticide molecules with light and heavy isotopes  
91 are degraded at slightly different rates, resulting in a kinetic isotope effect quantifiable  
92 by CSIA (Alvarez-Zaldívar et al., 2018; Elsayed et al., 2014; Melsbach et al., 2020;  
93 Schürner et al., 2016; Torabi et al., 2020). CSIA allowed to evaluate the bioavailability  
94 of pyrethroids in soil (Xu et al., 2018), and the degradation of  $\gamma$ -hexachlorocyclohexane  
95 (Qian et al., 2019) and polybrominated diphenyl ethers (Wang et al., 2020). Using  
96 CSIA, we recently demonstrated that biodegradation and sorption mainly drove  
97 dissipation of *S*-metolachlor and butachlor, respectively, in contrasted agricultural soils  
98 (Torabi et al., 2020). However, this study also brought up new questions. How do soil  
99 characteristics affect leaching, dissipation and biodegradation of anilide pesticides  
100 following rainfall events? What is the role of plants and the rhizosphere in the  
101 dissipation and biodegradation of anilide pesticides?

102 In the present study, we evaluated and compared the contribution of processes  
103 responsible for dissipation of anilide pesticides, including acetochlor (ACE), alachlor  
104 (ALA), *S*-metolachlor (SM), butachlor (BUT) and metalaxyl (MTY), in planted and  
105 unplanted soil mesocosms exposed to successive rainfall events. These five anilide  
106 pesticides were selected since they represent a major pesticide class used worldwide,  
107 although their applicability and toxicity largely vary. The herbicides acetochlor and  
108 alachlor are not allowed in agricultural crops in the EU, while *S*-metolachlor is most-  
109 used in the EU and South America, and butachlor in Asia (Torabi et al., 2020).  
110 Metalaxyl is used as a fungicide to control air- and soil-borne diseases in crops and  
111 vineyards (PPDB, 2019). While sharing the same general chemical structure (i.e., acyl  
112 derivatives of aniline), the anilide pesticides selected in this study display a broad  
113 spectrum of physico-chemical properties, which may drive their dissipation in soil-plant  
114 systems (see Supporting Information SI-A). Their dissipation in soil-plant systems have  
115 been found to be quicker in the rhizosphere than in the bulk soil (Bai et al., 2013;  
116 Elsayed et al., 2014), although they are usually studied individually. Two contrasted  
117 forest and vineyard soils were used to evaluate the effect of soil characteristics on  
118 pesticide dissipation processes using CSIA. We hypothesized that pesticide  
119 biodegradation would be larger in the planted mesocosms and in the soil solution that  
120 may leach after a rainfall event. In contrast, non-degradative processes were expected to  
121 predominate in non-planted mesocosms and in the bulk soil.

122

## 123 **2. Materials and Methods**

### 124 **2.1. Chemicals**

125 Anilide standards of acetochlor (ACE), alachlor (ALA), *S*-metolachlor (SM),  
126 butachlor (BUT) and metalaxyl (MTY) (PESTANAL, purities: 98%, 98%, 98%,96.9%

127 and 99.6%, respectively), metolachlor-*d*<sub>11</sub> (analytical grade purity: >97%),  
128 dichloromethane (DCM), acetonitrile (ACN) (HPLC grade, purity: >99.9%), and  
129 anhydrous magnesium sulfate (reagent grade: >97%) were all purchased from Sigma–  
130 Aldrich (St. Louis, MO, USA). Stock solutions of the pesticide mixture for soil spiking  
131 and standards for analysis were prepared as 1 g/L in DCM and ACN, respectively.  
132 Aliquots were stored at –20°C. Primary-secondary amine (PSA) was supplied by  
133 Supelco (Bellefonte, PA, USA).

134

## 135 **2.2. Experimental soils**

136 A vineyard and a forest soil with contrasted physicochemical characteristics  
137 were collected, respectively, on November 23 and 24, 2017, from two well-  
138 characterized sites. The vineyard soil was collected from a plot in the experimental  
139 catchment of Rouffach (Haut-Rhin, France, 47° 57' 43.0" N, 7° 17' 27.3" E, altitude: 200  
140 m asl) (Babcsányi et al., 2016; Duplay et al., 2014). The forest soil was collected from  
141 the Strengbach experimental catchment (Haut-Rhin, France, 48° 12' 57.9" N, 7° 11'  
142 52.5" E, altitude: 850 m asl) (Gangloff et al., 2016).

143 Sixty kg of topsoil were collected by random sub-sampling at each site with a  
144 distilled water- and ethanol-cleaned shovel. Sub-samples of vineyard topsoil (0-10 cm,  
145 Hor. A) were collected at ten locations across vine inter-rows from a 1486 m<sup>2</sup> plot  
146 managed under conventional wine-growing practices. Sub-samples of forested topsoil  
147 (0-10 cm, mostly organic horizon) were collected from eight different random locations  
148 of a spruces plot (1500 m<sup>2</sup>). Soil samples were transported in coolers into the  
149 laboratory, dried at 30°C with an air-forced oven and sieved using a 2-mm mesh.

150 After complete homogenization by mixing in quarters up to four times, an  
151 aliquot of each dried soil was weighed and dried at 105 °C until constant weight was

152 achieved to quantify the initial soil humidity. Soil physicochemical characteristics were  
153 measured following ISO methods and procedures, as detailed in SI-B.

154

### 155 **2.3. Mesocosms setup**

156 Two series of mesocosm experiments with pesticide-spiked soil, planted and  
157 unplanted, were set up in triplicate for each soil type (Figure 1). An additional two  
158 series of planted and unplanted mesocosms amended with sterile-filtered water without  
159 pesticides was run in parallel as nonspiked ‘blank’ experiments (data not shown). In  
160 total, twelve spiked mesocosm experiments and twelve nonspiked ‘blank’ experiment  
161 were followed in parallel.

162 Each soil mesocosms consisted of a rhizotron device, made of polypropylene  
163 plastic bottles with a nylon filter at the bottom (0.45  $\mu\text{m}$ ) and filled with 600 g of  
164 homogenized and dried vineyard or forest soil. Mesocosms were spiked with aqueous  
165 stock solutions containing ACE, ALA, SM, BUT and MTY. Soil bulk densities were  
166 2.0 and 0.7  $\text{g}/\text{cm}^3$  for vineyard and forest soils, respectively. Pesticide spiking in the  
167 aqueous solution was adjusted to 50% (W/W) of soil water content to ensure that both  
168 soil types reached water holding capacity (see SI-C for details). To homogenize water  
169 content and pesticide spiking (SI-D), sterile artificial rainwater (SI-E) was introduced by  
170 capillarity from the bottom of the mesocosms, while 100 mL of aqueous pesticide  
171 solution was added with a pipette at the top of the mesocosms. Final pesticide  
172 concentrations of each pesticide in the spiked soil mesocosms was 25 mg/kg,  
173 corresponding to severe environmental concentrations (Alvarez-Zaldívar et al., 2018;  
174 Lefrancq et al., 2017).

175 The mesocosms were placed randomly in a climate chamber (FDM LFC900SX  
176 PRO, Italy) and incubated for 75 days under controlled conditions of temperature (20



177 °C), humidity (70% *RH*) and photoperiodic lighting (16 h light, 8 h dark) with  
178 fluorescent LED lamps at 170  $\mu\text{mol photons/m}^2$ .

179

#### 180 **2.4. Mesocosm operations**

181 Each of the planted mesocosms were seeded with 320-340 Italian ryegrass  
182 (*Lolium multiflorum* sp.) at 1-2 seeds per  $\text{cm}^2$  (Bourceret et al., 2015). Ryegrass was  
183 chosen since it is an herbaceous fast-growing species widely used to cover vineyard  
184 soils (Fernández-Calviño et al., 2017). Mesocosms without pesticides were incubated as  
185 parallel duplicate control mesocosms to determine the necessary incubation time to  
186 reach the vegetation maturity stage.

187 The soil water content of the mesocosms was controlled by weighing daily each  
188 mesocosm and adjusted if necessary. A soil water content of 50% (*W/W*) was  
189 maintained across the experiment by spraying MilliQ<sup>®</sup> water on the top of the  
190 mesocosms.

191 The preparation of artificial rainfall and the rainfall simulator system are  
192 described in SI-E. Artificial rainfall events consisted of a total volume of 300 mL,  
193 corresponding to a rainfall depth of 15 mm, applied on the mesocosms with a duration  
194 of 12 min at 62 mm/h. This rainfall event pattern represents a medium duration event  
195 with intermediate intensity in a temperate climate (Meite et al., 2018). A first artificial  
196 rainfall (R1) was applied when plants in control nonspiked mesocosms reached the  
197 elongation stage (i.e., 45 days after seeding). The second and third artificial rainfalls  
198 (R2, R3) were applied 15 (day 60) and 30 days after R1 (day 75), respectively.

199

#### 200 **2.5. Mesocosms sampling**

201           The sampling scheme and sample preparation procedures are detailed in SI-F.  
202   One day after pesticide spiking (day 1), triplicate soil sub-samples were collected  
203   randomly from each mesocosm. Then, they were homogenized and pooled separately  
204   per experimental condition. Leachate samples were collected after each artificial rainfall  
205   on days 45, 60 and 75, denoted as L45, L60 and L75, respectively. Leachates were  
206   sampled separately from each mesocosm. Half of the leachate volume was immediately  
207   filtered using a 0.45 µm glass fibre filter for pesticide analysis. The other half was  
208   filtered using a 0.45 µm cellulose acetate filter for pH, DOC, DIC, major anions and  
209   cations and minor nutrient analysis. For each condition, one mesocosm (day 45) and  
210   duplicate mesocosms (day 75) were sacrificed to collect bulk soil, rhizosphere, plant  
211   aerial parts and roots.

212

## 213   **2.6. Pesticide quantification and CSIA**

214           Pesticides were extracted from soil and rhizosphere samples using a solvent  
215   extraction and cleanup procedure adapted from Ivdra et al. (2014). Pesticides were  
216   extracted from leachates following a protocol adapted from US EPA Method 525.2 and  
217   described by Elsayed et al. (2014). Briefly, a SPE (solid phase extraction) procedure  
218   was followed, using SolEx C18 cartridges (1 g, Dionex®, CA, USA) on an AutoTrace  
219   280 SPE system (Dionex®, CA, USA). Concentrations of ACE, ALA, SM, BUT and  
220   MTY from soils and leachates were measured using GC/MS as described in SI-G and  
221   SI-H.

222           The carbon isotope compositions ( $\delta^{13}\text{C}$ ) of ACE, ALA, SM, BUT and MTY and  
223   nitrogen ( $\delta^{15}\text{N}$ ) of SM were analysed using a GC-C-IRMS system consisting of a  
224   TRACE™ Ultra Gas Chromatograph (ThermoFisher Scientific) coupled via a GC  
225   IsoLink/Conflow IV interface to an isotope ratio mass spectrometer (IRMS DeltaV

226 Plus, ThermoFisher Scientific) configured as described in Masbou et al. (2018) and  
227 detailed in the SI-I.

228 Every injection was checked to ensure that it remained within the linearity  
229 ranges (SI-I). A set of in-house BTEX (for C-CSIA), IAEA-600 caffeine (for N-CSIA),  
230 and pesticide standards with known isotopic composition, determined using an  
231 elemental analyser-isotopic ratio mass spectrometer (Flash EA IsoLinkTMCN IRMS,  
232 Thermo Fisher Scientific, Bremen, Germany) was measured at least every ten injections  
233 to control analytical reproducibility and accuracy of measurements.

234 Extraction and measurement methods used for pesticide CSIA from soil and  
235 leachate samples did not cause significant isotope fractionation ( $\Delta\delta^{13}\text{C}\leq 1\text{‰}$  and  
236  $\Delta\delta^{15}\text{N}\leq 0.5\text{‰}$ ) (SI-I, J and K). Analytical uncertainty of 0.5 ‰ and 0.7 ‰ was attributed  
237 to each measurement based on  $\pm 1$  SE of, respectively, mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values from  
238 triplicate measurements for each sample, and the long-term reproducibility  
239 measurements.

240

## 241 **2.6. Data analysis**

### 242 **2.6.1. Water budget, nutrient and pesticide leaching**

243 Water mass budget (Thorntwaite and Mather, 1955) was calculated as follows:

$$244 \quad P + SWC = ETP + L \quad (1)$$

245 where  $P$  corresponds to precipitation and  $SWC$  to soil water content or the water mass  
246 per mass of dry soil.  $ETP$  and  $L$  correspond to potential evapotranspiration from planted  
247 mesocosms and to leachates, respectively.

248 The mass water budget for each compartment was used to estimate the pesticide  
249 exports following each rainfall event.

250 Pesticide loads ( $\mu\text{g}$ ) in leachates were estimated from individual pesticide  
 251 concentrations  $\text{conc}_t$  (in  $\mu\text{g/L}$ ) in leachates (L45, L60 and L75), and the leached volume  
 252 ( $V_t$ , in  $L$ ) per mesocosm at each sampling time  $t$ :

$$253 \text{ Pesticide loads } [\mu\text{g}] = \text{conc}_t \times V_t \quad (2)$$

254 The leachate export, i.e., the proportion of pesticide mass leached from a  
 255 mesocosm with respect to the initial amount of spiked pesticides, and the first flux  
 256 contribution, i.e., the contribution of the first leachate (L45) to the total pesticide export  
 257 by leaching, were calculated according to Eq. 3 and 4, respectively:

$$258 \text{ Leachate export (LE) } [\%] = \frac{\text{pesticide leached}_{\Sigma t}}{\text{pesticide}_{\text{initial}}} \times 100 \quad (3)$$

$$259 \text{ First flux contribution (FFC) } [\%] = \frac{\text{pesticides leached}_{t45}}{\text{pesticides leached}_{\Sigma t}} \times 100 \quad (4)$$

260 where  $\text{pesticides leached}_{\Sigma t}$  is the total mass of pesticide exported during the  
 261 experiment,  $\text{pesticide}_{\text{initial}}$  is the mass spiked of each pesticide, and  
 262  $\text{pesticides leached}_{t45}$  is the pesticide mass in L45.

263

### 264 **2.6.2. Quantification of pesticide dissipation**

265 Pesticide dissipation in each mesocosm, accounting for the difference between  
 266 the initial amount of the spiked pesticides and the losses by leaching, degradation, plant  
 267 uptake and ageing in the soil and rhizosphere, was estimated using Eq. 5:

$$268 \text{ Dissipation } [\%] = \frac{m_{\text{soil},t1} - m_{\text{soil},t}}{m_{\text{soil},t1}} \times 100 \quad (5)$$

269 where  $m_{\text{soil},t1}$  and  $m_{\text{soil},t}$  represent pesticide mass [ $\mu\text{g}$ ] at the initial sampling time ( $t1$ )  
 270 and time  $t$  (45, 75) in soil mesocosms, respectively.

271

### 272 **2.6.3. Dissipation kinetics**

273 Pesticide data in each condition were fitted to an exponential decay single first-  
274 order (SFO) model according to NAFTA guidelines (USEPA). Dissipation rates ( $k$ )  
275 were estimated when the model provided a significant fit ( $R^2 > 0.6$  and  $p < 0.05$ ) from  $n$   
276  $\geq 3$  points (SI-N).

277

#### 278 **2.6.4. Carbon stable isotope notation and calculation**

279  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were normalized by the Vienna Pee Dee Belemnite  
280 (VPDB) standard for carbon and by the international AIEA600 air standard for nitrogen  
281 as follows:

$$\delta^h X[\text{‰}] = 1000 \cdot \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \quad (1)$$

282 where  $\delta^h X$  is expressed in per thousand (‰) and  $R$  refers to the ratio of heavy ( $h$ ) to  
283 light ( $l$ ) isotopes of the element  $X$   $\left( \frac{h_X}{l_X} \right)$  in the analysed samples and the international  
284 standards. Samples were injected in triplicate, and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values are reported  
285 as the associated arithmetic mean. Each measurement was checked to remain within the  
286 linearity ranges for C and N. A set of in-house BTEX (for C), caffeine (AIEA 600, for  
287 N) and pesticide (for C and N) standards with known isotopic composition (determined  
288 by EA-IRMS) was measured at least every ten injections to control the measurement  
289 quality. Reference  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  compositions of BTEX and pesticide standards were  
290 determined at our isotope facility using an elemental analyser-isotopic ratio mass  
291 spectrometer (Flash EA IsoLinkTMCN IRMS, Thermo Fisher Scientific, Bremen,  
292 Germany).

293 The average isotope value of the residual nondegraded fraction of herbicides  
294 varies according to the Rayleigh equation:

$$295 \quad \frac{(^{13}\text{C}/^{12}\text{C})_t}{(^{13}\text{C}/^{12}\text{C})_0} = \frac{\delta^{13}\text{C}_{t+1}}{\delta^{13}\text{C}_{0+1}} = f^\varepsilon \quad (7)$$

296 where  $f$  is the residual substrate fraction,  $\delta^{13}\text{C}_t$  the corresponding isotope values at time  $t$   
 297 and  $\delta^{13}\text{C}_0$  is the initial isotope value for carbon of each compound at the beginning of the  
 298 experiment ( $f = 1$ ).

299 The isotopic fractionation factor  $\varepsilon$  relates changes of isotope ratios to the extent  
 300 of transformation. The  $\varepsilon_c$  factor was determined based on carbon isotope data obtained  
 301 across the experiment (days 1 - 75) using a linear regression according to:

$$302 \quad \ln \frac{\delta^{13}\text{C}_{t+1}}{\delta^{13}\text{C}_{0+1}} = \varepsilon \ln f \quad (8)$$

303 where  $\delta^{13}\text{C}_0$  and  $\delta^{13}\text{C}_t$  are carbon isotope ratios for each compound studied, and  $C_0$  and  
 304  $C_t$  are concentrations at time 0 (day 1) and  $t$ , respectively. Values of carbon isotopic  
 305 fractionation  $\varepsilon_c$  for the pesticides were determined if (i) isotope fractionation during the  
 306 incubation period was significant ( $\Delta\delta^{13}\text{C} > 2\text{‰}$ , Álvarez-Zaldívar et al., 2008), (ii) the  
 307 linear regression of the Rayleigh plot was significant ( $R^2 > 0.6$ ,  $P < 0.05$ ), and (iii)  $n \geq 3$   
 308 sampling times were available.

309

### 310 **2.6.5. Quantification of pesticide biodegradation**

311 The extent of biodegradation ( $B_C$ ) of all compounds studied at time  $t$  in planted  
 312 and unplanted mesocosms was estimated from  $\delta^{13}\text{C}$  values obtained in the present study  
 313 using the inverse form of the Rayleigh equation (Elsner et al., 2007). Isotopic  
 314 fractionation factors ( $\varepsilon_c$ ) of anilide pesticides retrieved from Elsayed (2014) for ACE (-  
 315  $3.5 \pm 0.5$ ) and ALA ( $-2.0 \pm 0.3$ ), Masbou et al., (2018a, 2018b) for BUT ( $-3.7 \pm 0.7$ ) and  
 316 MTY ( $-2.0 \pm 1.3$  in F and  $-0.9 \pm 0.5$  in V soil), respectively and Álvarez-Zaldívar et al.  
 317 (2018) for SM ( $-1.5 \pm 0.5$ ) were used to estimate  $B_{C,t (CSIA)}$ , since the experiments were  
 318 carried out in biotic mesocosms with the same or similar soils:

$$B_{C,t(CSIA)} = 1 - f = 1 - \left[ \frac{(1000 + \delta^{13}C_t + \Delta\delta^{13}C_t)}{1000 + \delta^{13}C_0} \right]^{1000/\epsilon} \quad (9)$$

320

321

322

323

### 324 **3. Results and discussion**

#### 325 **3.1. Water balance, plant growth and soil characteristics**

326 Water leaching accounted for  $75 \pm 3\%$  ( $\bar{X} \pm \sigma$ ) and  $82 \pm 2\%$  of the initial rainfall in  
 327 planted and unplanted vineyard mesocosms, and for  $34 \pm 4\%$  (planted and unplanted) in  
 328 forest mesocosms (SI-L).

329 Plant growth started in spiked planted microcosms from day 45 onwards, which  
 330 correlated with the first rainfall. At day 75, plant biomass in spiked and nonspiked  
 331 planted mesocosms was, respectively, 0.1 and 26.8 g/kg soil (dry aerial part). The  
 332 length of plant aerial parts ranged from 3-5 and 15-20 cm, respectively, while roots  
 333 ranged over a length of 1-4 and 12 cm respectively, with a density of  $1.1 \cdot 10^{-3}$ - $5.5 \cdot 10^{-4}$   
 334 and  $2.2 \cdot 10^{-2}$ - $6.7 \cdot 10^{-3}$  g (dry weight)/cm<sup>3</sup> soil, respectively, emphasising the suppressing  
 335 effect of pesticides on plant growth in spiked mesocosms.

336 Plants contributed to decrease pH in vineyard soil due to nutrient uptake and  
 337 leaching of basic nutrients. Leachate pH values from vineyard mesocosms were slightly  
 338 lower in planted mesocosms (7.2-7.5) than in unplanted mesocosms (7.6-7.8), although  
 339 soil pH values remained constant over the experiment (planted: 7.8-8.0, unplanted: 8.0-  
 340 8.3) (Table 1). In contrast, the forest soil pH (initial pH = 3.4) increased in both planted  
 341 (up to 4.0) and unplanted (up to 3.8) mesocosms during the experiment, whereas the pH  
 342 of leachates varied from 3.4 to 3.6 in both types of mesocosms. Acid root exudates  
 343 during plant growth may displace major cations such as K or Mg in the soil solution,  
 344 which likely increased the pH of the acidic forest soil (Canarini et al., 2019) (SI-B).

345 Leaching of dissolved organic carbon (DOC) was 2-fold higher in the forest than  
346 in the vineyard mesocosms (Table 1), which can be related to the higher OM content in  
347 forest soil (SI-B). However, DOC concentrations in leachates from unplanted  
348 mesocosms were higher than leachates from planted mesocosms for both soil types,  
349 suggesting higher DOC retention in planted mesocosms during plant growth, and  
350 distinct carbon turnover in planted and unplanted mesocosms. Cation exchange capacity  
351 (CEC) was slightly higher in the vineyard than in the forest mesocosms (Table 1),  
352 reflecting larger cation uptake from the soil matrix, especially after pesticide leaching,  
353 which might contribute to the larger plant growth in the vineyard mesocosms.

354

### 355 **3.2. Pesticide leaching**

356 Pesticide leaching from the vineyard mesocosms was generally higher than from  
357 the forest mesocosms (Figure 2). The contribution of the first rainfall event to pesticide  
358 export by leaching ranged from 69 up to 99% (Table 2). The first rainfall event  
359 following pesticide application thus exported the largest fraction of pesticides, as  
360 previously observed with synthetic (Hunsche et al., 2007) and Cu-based fungicides  
361 (Pérez-Rodríguez et al., 2015; Pérez-Rodríguez et al., 2016). The pesticide  
362 concentrations in leachates normalized per kg of mesocosm soil varied from  $1 \cdot 10^{-3}$   
363 mg/kg (ACE) up to 3.3 mg/kg (MTY) (Figure 2).

364 Pesticide leaching from the unplanted vineyard mesocosms was larger than  
365 leaching from the planted mesocosms, whereas leaching was similar in the planted and  
366 unplanted forest mesocosms. However, pesticide leaching from both soil types, ordered  
367 from highest to lowest, followed a  $MTY > SM \approx BUT > ALA \approx ACE$  pattern (Figure  
368 2).



369 MTY leaching exports accounted for up to 12% in unplanted and 7% of initially  
370 spiked MTY in planted vineyard mesocosms, whereas it remained <3% in forest  
371 mesocosms (Table 2). The relatively high leaching of MTY in both vineyard and forest  
372 mesocosms reflects the high solubility (SI-A) and low sorption propensity of MTY  
373 (Fernández-Calviño et al., 2015). However, leaching exports were in the range of those  
374 established by the EU (EFSA, 2015). Lower leaching exports of MTY in the planted  
375 mesocosms (Table 2) suggests that plants enhanced MTY retention and/or  
376 biodegradation, possibly by sustaining rhizosphere processes associated with MTY  
377 transformation (Baker et al., 2010).

378 Leaching of SM from vineyard unplanted mesocosms (8% in total) was three  
379 times higher than leaching of ACE and ALA. However, leaching exports of SM, ACE  
380 and ALA were similar in planted mesocosms (3%), emphasizing the effect of plants on  
381 SM retention in vineyard mesocosms. SM and BUT leaching from forest mesocosms  
382 remained <1.4%, especially in unplanted mesocosms (Table 2). ALA leaching exports  
383 from vineyard and forest mesocosms remained <3% and <2%, respectively, across the  
384 experiment (Table 2). Leaching exports of ALA were similar to those in soils with  
385 higher SOM and acidic pH (Heyer and Stan, 1995) and in Baltic soils with low organic  
386 matter (Sakaliene et al., 2007) (ranging from 0.6 to 1.5%) but were lower than those  
387 found in tropical soils (about 6%) from Brazil (Giori et al., 2014). These differences  
388 underscore that soil properties affected ALA partitioning and its concentration in the  
389 soil solution. Similarly, ALA, ACE leaching in vineyard mesocosms was higher in  
390 unplanted (3%) than in planted vineyard mesocosms (1%), whereas it accounted for  
391 <1% in forest mesocosms.

392 Larger pesticide leaching in vineyard than in forest mesocosms stems from a  
393 combination of soil characteristics, including soil texture, hydraulic retention time

394 (HRT), water holding capacity (WHC) and pesticide mobilization. The compaction of  
395 the clay-loam soil in vineyard mesocosms led to water ponding during rainfall events,  
396 as observed previously with the same soil (Meite et al., 2018). Water ponding in  
397 vineyard mesocosms reduced the water infiltration rate while increasing the HRT and  
398 soil-water interactions compared to forest mesocosms. A longer HRT and soil-water  
399 interaction time favoured soil dispersion and thus pesticide remobilisation. This,  
400 together with a limited WHC ( $34 \pm 3.5\%$ , see SI-C), increased pesticide leaching from  
401 vineyard mesocosms. In contrast, and despite a sand-loam texture reducing the HRT of  
402 the forest soil, a higher organic matter content and WHC ( $48 \pm 0.8\%$ , see SI-C), as well  
403 as the type of clay, mainly 2:1 with larger size of interlayer spaces, limited pesticide  
404 leaching from forest mesocosms (SI-M).

405

### 406 **3.3. Pesticide dissipation**

407 Pesticides could be quantified in the bulk and rhizosphere soil samples, whereas  
408 low root and biomass development hampered pesticide quantification in the plants.  
409 Pesticide dissipation up to day 45 ranged from 30 to 88% in the vineyard and forest  
410 mesocosms and was similar in planted and unplanted mesocosms (Figure 3). However,  
411 pesticide dissipation mostly increased in planted mesocosms from day 45 onwards,  
412 particularly in forest mesocosms (Figure 3), reaching up to 99% dissipation on day 75,  
413 indicating that plant growth observed from day 45 onwards favoured pesticide  
414 dissipation (data not shown). Pesticides suppressed plant growth before day 45, which  
415 limited the influence of plants on pesticide dissipation up to day 45. The first rainfall on  
416 day 45 washed off 69 to 99% of the total amount of leached pesticide (Figure 3), which  
417 reduced the herbicide inhibition effect. Less pesticide in the soil mesocosms allowed  
418 plant growth from day 45 onwards with concomitant rhizosphere development.

419 Pesticide dissipation patterns were similar in the vineyard and forest mesocosms,  
420 and globally ordered from lowest to highest solubility in water, as follows: BUT > ACE  
421 > ALA  $\approx$  SM > MTY (SI-A).

422 Total dissipation of BUT, including leaching, was similar, although slightly  
423 higher in vineyard (>98%,  $k > 0.17 \pm 0.17 \text{ day}^{-1}$ ) than in forest mesocosms (>96%,  $k =$   
424  $0.09 \pm 0.09 \text{ day}^{-1}$ ) (Figure 3 and Table S5, SI-N). The BUT dissipation, even when the  
425 contribution of leaching was considered, was faster in the vineyard planted mesocosms  
426 than previous estimations using the same soil (Torabi et al., 2020). In contrast,  
427 dissipation in forested soil was like the dissipation in a paddy soil with similar SOM  
428 content studied by Torabi et al. (2020). Fast BUT dissipation can be related to its high  
429  $K_{oc}$  and lower water solubility, and sorption was likely the main process affecting  
430 apparent BUT dissipation in mesocosm experiments (Gevao et al., 2000; Torabi et al.,  
431 2020).

432 Dissipations of ACE and ALA was >98% and >90% in the vineyard and the  
433 forest mesocosms, respectively, and was similar in both planted and unplanted  
434 mesocosms (Figure 3 and Table S5, SI-N). Dissipation kinetics in the vineyard  
435 mesocosms are in agreement with typical half-lives ( $DT_{50}$ ) – soil values (SI-A), as well  
436 as previous observations in paddy soils (Yu et al., 2019) and in multi-contaminated soils  
437 with similar physicochemical properties (Jiang et al., 2018). Significantly lower half-  
438 lives in the rhizosphere than in the bulk soil were found for ACE (Bai et al., 2013),  
439 suggesting that degradation is more extensive in the planted mesocosms.  $DT_{50}$  values  
440 for ALA inferred from vineyard mesocosms were similar to reference values, while the  
441 values for forest mesocosms were similar to those reported in field studies and higher  
442 than reference values (up to 21 days, University of Hertfordshire, 2019) (SI-A), also  
443 reflecting the higher affinity of ALA to forest SOM compared to ACE (ALA  $K_{OC} >$

444 ACE  $K_{OC}$ ). Lower ACE and ALA dissipation in forest than in vineyard mesocosms was  
445 consistent with slower dissipation rates (Table S5, SI-N) and with results of a simulated  
446 organic matrix biobed experiment with high organic matter content (Chu and Eivazi,  
447 2018).

448 Similarly, total dissipation of SM was >96% (Figure 3; Table S5, SI-N), slightly  
449 higher in planted than in unplanted mesocosms, and higher than in previous studies for  
450 the same soil (Torabi et al., 2020). Higher dissipation compared to previous studies  
451 reflects the contribution of SM leaching to total dissipation and suggests that plants  
452 enhanced SM dissipation. Dissipation rates were slower in the forest (Table S5, SI-N)  
453 than in the vineyard mesocosms, and corresponded to longer half-lives (33 to 51 d) than  
454 the dissipation rates observed in an acidic soil with similar physicochemical properties  
455 (Gluhar et al., 2019).

456 Total MTY dissipation was larger than 97% and 58% in the vineyard and the  
457 forest mesocosms, respectively (Figure 3), and dissipation rates were faster in the  
458 vineyard (0.04-0.05 d<sup>-1</sup>) than in the forest (0.01 d<sup>-1</sup>) mesocosms (Table S5, SI-N).  
459 Slower MTY dissipation in forest mesocosms is consistent with MTY half-lives in soils  
460 at pH lower than 7.5 (Sukul and Spiteller, 2001) or more acidic (Buerge et al., 2003).

461 Pesticide dissipation in mesocosms resulted from leaching of the soil solution  
462 following a rainfall event, as well as pesticide ageing and biodegradation in the bulk soil  
463 and the rhizosphere. Overall, pesticides and soil physicochemical characteristics (SI-A)  
464 could be related to pesticide dissipation patterns in the mesocosms, with generally lower  
465 dissipation in the forest than in the vineyard mesocosms (Fig. 3). Higher organic matter  
466 content (5.3%) and, putatively, the type of clay (2:1 abundance) in forest soil enhanced  
467 pesticide sorption due to the availability of more sorption sites with larger sizes of  
468 interlayer spaces. Such conditions can limit pesticide availability, and thus pesticide

469 biodegradation and export. In addition, lower pH of the forest soil can increase pesticide  
470 half-lives (Baker et al., 2010) and affect microbial communities (Fernández-Calviño and  
471 Bååth, 2010; Rousk et al., 2010), which in turn can influence pesticide biodegradation.

472         However, pesticide distribution and dissipation differed not only among  
473 pesticides but also between planted and unplanted mesocosms (Table 2 and Figure 3).  
474 Our results also support the idea that the rhizosphere is a major hotspot of pesticide  
475 dissipation in planted systems (Kuzyakov and Blagodatskaya, 2015). Pesticide  
476 biodegradation in the rhizosphere can be quick (Kuzyakov and Razavi, 2019), and  
477 rhizosphere processes can influence a large volume of surrounding soil (Dennis et al.,  
478 2010). However, the presence of plants limited pesticide leaching in the vineyard and  
479 forest soils, which emphasizes the role of the rhizosphere on water and pesticide transfer  
480 in the plant-soil system (Hinsinger, et al., 2005; Hinsinger, et al., 2009). Lower  
481 quantities of pesticides were found in the rhizosphere, and more generally the planted  
482 mesocosms, than in the bulk soil. The well-developed rhizosphere in vineyard  
483 mesocosms might have favoured the microbial biomass and specific microorganisms  
484 involved in pesticide degradation (Bourceret et al., 2015). In contrast, larger SOM and  
485 different clays structures in the forest mesocosms increased pesticide sorption and  
486 residence time (Giori et al., 2014), which limited pesticide leaching.

487         Overall, our results emphasise that plants and their rhizosphere influenced  
488 anilide pesticide dissipation, and putatively biodegradation, which is further discussed  
489 below using CSIA data.

490

491         **3.4. Insights into anilide pesticide biodegradation in soil mesocosms by**  
492 **CSIA**

493 CSIA of pesticides was carried out in leachate and bulk soil samples, whereas  
494 pesticide amounts were insufficient for CSIA in the aerial parts of plants and  
495 rhizosphere soil samples.

496 In bulk soil, changes in  $\delta^{13}\text{C}$  values were less pronounced than in leachates and  
497 only occurred only at day 75 ( $\Delta\delta^{13}\text{C}$  up to  $8.2 \pm 0.8\text{‰}$  for ACE in bulk soil of planted  
498 vineyard mesocosms at day 75) (Figure 4). The absence of significant isotope  
499 fractionation of SM, BUT and MTY in the bulk soil before 75 days ( $\Delta\delta^{13}\text{C} < 2\text{‰}$ , within  
500 the range of analytical uncertainty) suggests that sorption and leaching contributed more  
501 to SM, BUT and MTY dissipation in bulk soil than biodegradation. Based on  
502 physicochemical properties of the pesticides (SI-A) and mass balance (Figure 3), the  
503 contribution of sorption was higher for SM and BUT than for ACE and ALA, whereas  
504 leaching was the main MTY dissipation process, limiting the role of biodegradation of  
505 SM, BUT and MTY although dissipation apparently occurred.

506 In contrast, biodegradation dominated over other dissipation processes in the soil  
507 solution of both the vineyard and the forest mesocosms from day 45 onwards.  
508 Significant isotope fractionation observed in leachates from the mesocosms indicated  
509 the occurrence of *in situ* degradation (Figure 4A). Carbon isotope composition ( $\delta^{13}\text{C}$ ) of  
510 pesticides in leachates changed significantly following rainfall events on days 60 and  
511 75, with changes of isotopic ratios ( $\Delta\delta^{13}\text{C}$ ) up to  $13.9 \pm 0.5\text{‰}$  and  $12.5 \pm 0.8\text{‰}$  (ALA) in  
512 the planted vineyard and forest mesocosms, respectively (Figure 4A). Overall, isotopic  
513 fractionation of pesticides was significantly lower in both the planted and unplanted  
514 forest mesocosms than in vineyard mesocosms ( $p < 0.05$ ). Isotopic fractionation factors  
515  $\epsilon_{\text{C}}$  that relate changes of isotope ratios to the extent of transformation (Elsner et al.,  
516 2005) were determined for ACE and ALA, using the isotopic fractionation factors cited  
517 previously, since good fitting with the Rayleigh model was observed ( $R^2 > 0.6$ ,  $p < 0.05$ ;

518  $n < 5$ ). The values of  $\epsilon_C$  obtained for ACE and ALA with Eq. 8 ranged from  $-0.8 \pm 0.4\%$   
519 (ACE in the planted forest mesocosms) up to  $-1.5 \pm 0.6\%$  (ALA in the vineyard  
520 mesocosms), which were slightly higher than the values of  $\epsilon_C$  obtained previously in  
521 Elsayed et al., (2014) in a wetland mesocosms and significantly higher than the values  
522 of  $\epsilon_C$  obtained during abiotic hydrolysis by Masbou et al., (2018a).  $\epsilon_C$  values did not  
523 differ significantly between planted and unplanted vineyard and forest mesocosms  
524 ( $p < 0.05$ ). The extent of biodegradation in leachates from the vineyard and the forest  
525 mesocosms for ACE, ALA, and SM, estimated on day 75 based on isotopic data (Eq. 9),  
526 reached 93, 98 and 95 to 100%, respectively (Table 3).

527 Another important finding is that biodegradation was more pronounced in  
528 leachates from planted than from unplanted mesocosms (Table 3). Changes in  $\delta^{13}\text{C}$   
529 values in leachates after 45 days were larger for ACE, ALA, SM and BUT in the  
530 vineyard planted mesocosms ( $\Delta\delta^{13}\text{C}$  up to  $13.9 \pm 0.5\%$  for ALA at day 75) than in  
531 leachates from unplanted mesocosms and in the bulk soil (Figure 4). In leachates from  
532 the forest planted mesocosms, changes in  $\delta^{13}\text{C}$  values increased over time and were  
533 larger ( $\Delta\delta^{13}\text{C}$  up to  $9.1 \pm 0.4$  for ACE) than in unplanted mesocosms ( $\Delta\delta^{13}\text{C}$  up to  $4.4 \pm$   
534  $0.5\%$  for ACE) on day 75 (Figure 4).

535 Overall, biodegradation was identified as the major process responsible for  
536 ACE, ALA and SM dissipation in the soil solution from both vineyard and forest  
537 mesocosms. Biodegradation occurred mainly in the soil solution where pesticides are  
538 mostly available to microorganisms (Megharaj et al., 2011). Despite the lack of sterile  
539 experiments in this study, abiotic degradation of pesticides in the mesocosms are likely  
540 minor, especially under neutral/acidic conditions where the anilide pesticides were  
541 shown to be stable to hydrolysis (EXTOXNET, 2000; Zheng and Ye, 2001; Masbou et

542 al., 2018a). Hence, we assumed that observed changes in the carbon isotopic  
543 composition of pesticide in the mesocosms reflected *in situ* biodegradation.

544 Nondegradative processes, including loss by leaching and sorption, also  
545 contributed to SM, BUT and MTY dissipation in the bulk soil of vineyard and forest  
546 mesocosms. ACE and ALA were biodegraded mainly in the leachates from the vineyard  
547 mesocosms, while biodegradation contributed to the dissipation of all pesticides except  
548 BUT in the leachates from the forest mesocosms (Table 3). This also highlights the  
549 potential of CSIA to differentiate dissipation processes in soil, by estimating the  
550 contribution of pesticide biodegradation versus pesticide sorption. Pesticide CSIA based  
551 on carbon alone may, however, not be sufficient to distinguish different pathways of  
552 transformation of pesticides. Multielement CSIA (ME-CSIA) of anilide pesticides ( $\delta^2\text{H}$ ,  
553  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{37}\text{Cl}$ ) could be applied for this purpose.

554 Here only few leachate samples satisfied the requirements for reliable  $\delta^{15}\text{N}$   
555 measurements of SM in the vineyard mesocosms. Changes in the  $\delta^{15}\text{N}$  values for SM  
556 were similar in the planted ( $\Delta\delta^{15}\text{N}$  up to  $2.8 \pm 0.7\text{‰}$  on day 75) and the unplanted  
557 ( $\Delta\delta^{15}\text{N}$  up to  $2.9 \pm 0.7\text{‰}$  on day 75) mesocosms (SI-O). As an attempt to examine the  
558 degradation mechanisms of SM in the mesocosms, a dual-isotope plot of the changes in  
559 isotope ratios between N and C ( $\Delta\delta^{15}\text{N}$  vs.  $\Delta\delta^{13}\text{C}$ ) was drawn to retrieve the  $\Lambda$  values  
560 from the slopes (SI-O). Distinct  $\Lambda$  values suggest different biodegradation mechanisms  
561 in the planted ( $\Lambda = \Delta\delta^{15}\text{N}/\Delta\delta^{13}\text{C} = 0.7 \pm 0.6$ , 95% CI,  $n=4$ ) and the unplanted ( $\Lambda = 1.8 \pm$   
562  $0.8$ , 95% CI,  $n=4$ ) mesocosms. Interestingly, the  $\Lambda$  value retrieved from the planted  
563 mesocosm was like the  $\Lambda$  value previously observed during SM hydrolysis ( $\Lambda = 0.8 \pm$   
564  $0.7$ ; Masbou et al., 2018a). Although these results emphasize the potential of ME-CSIA,  
565 identification of the SM degradation pathways based on transformation products and the  
566 associated C and N isotope fractionation is required to interpret the degradation



567 mechanisms of SM in soil-plant systems. Currently and due to the above-mentioned  
568 limitations, the contribution of carbon to the molar mass of anilide pesticides makes  
569 carbon a reliable candidate to evaluate in the routine the occurrence of biodegradation  
570 using CSIA at typical low (sub-mg/L) concentrations of pesticides in soil-plant systems.  
571 However, when the reaction involves the breakage of a bond containing chlorine as a  
572 single heteroatom in the pesticide molecule (e.g., the C-Cl bond for the biotic or abiotic  
573 hydrolysis of metolachlor), the expected strong Cl fractionation may be more  
574 informative than carbon to evaluate *in situ* degradation (Ponsin et al., 2019). In addition,  
575 Cl-CSIA for anilide herbicides with GC-MS typically requires smaller amounts of  
576 compounds than for C-CSIA with GC-IRMS.

577         Biodegradation was generally more pronounced in leachates from planted than  
578 from unplanted mesocosms. Plants can enhance pesticide sorption on SOM or/and  
579 minerals within the rhizosphere, while root exudates favour pesticide desorption and  
580 availability, thereby increasing biodegradation (Huang et al., 2016; Lefevre et al., 2013;  
581 Violante and Caporale, 2015). While microbial (co-)metabolism is a major degradation  
582 processes for anilide pesticides (Elsayed et al., 2014; Imfeld et al., 2018), the presence  
583 of a plant-root system in the soil can sustain rhizosphere microbial communities through  
584 root exudates, mainly consisting of labile organic matter (Mommer et al., 2016). We  
585 hypothesized that the plant-root system altered the carbon/nitrogen balance in the  
586 rhizosphere and affected the microbial ability to use carbon (Jones et al., 2009), and  
587 rhizosphere microbial communities were associated with anilide degradation. These  
588 processes appear to be decoupled from the bulk soil and the absence of plants may limit  
589 the microbial degradation functions of soil (Kuzyakov and Blagodatskaya, 2015),  
590 although our results suggest that biodegradation in bulk soil of planted mesocosms was  
591 more pronounced than in the unplanted mesocosms.

592

#### 593 **4. Conclusions**

594           The leaching and dissipation of five widely used anilide pesticides in planted  
595 and unplanted vineyard and forest soil mesocosms were investigated and compared.  
596 Analysis of dissipation kinetics, leachate export and CSIA of the pesticides allowed the  
597 evaluation of the contribution of degradative and non-degradative processes dissipation  
598 in soil and the leachates of planted and unplanted mesocosms and for two contrasted  
599 vineyard and forest soils. The results indicate that both degradative and nondegradative  
600 processes contributed to the apparent dissipation of BUT, SM, ALA, ACE and MTY in  
601 planted and unplanted mesocosms. The contribution of leaching, ageing in soil and  
602 biodegradation varied, depending mainly on the physicochemical characteristics of the  
603 pesticides, the SOM content and the occurrence of plants. High affinity of pesticide to  
604 SOM and clays may increase sorption, whereas pesticide degradation was enhanced by  
605 plants and their rhizosphere, which may increase pesticide bioavailability and  
606 desorption and eventually favour pesticide leaching.

607           Most importantly, this study shows the potential of CSIA to evaluate the  
608 occurrence and extent of biodegradation of pesticide mixtures in plant-soil systems.  
609 Although physicochemical characteristics of the pesticides studied differed  
610 significantly, our approach showed significant pesticide biodegradation in the soil  
611 solution following rainfall events rather than in the bulk soil, which reflected their  
612 bioavailability of the pesticides. However, the contribution of leaching to the total  
613 dissipation of pesticide remained limited, and pesticide biodegradation depended mainly  
614 on the physicochemical characteristics of soil and pesticides.

615           In conclusion, this study proposes an approach to tease apart the dissipation  
616 processes of anilide pesticides in the soil-plant system. In the future, a similar approach

617 may be adapted to various soil-plant systems and at different scales to examine realistic  
618 micropollutant dissipation scenarios accounting for *in situ* biodegradation and the role  
619 of rhizosphere.

620

## 621 **Acknowledgements**

622 Paula Pérez-Rodríguez was supported by a Postdoctoral fellowship from the  
623 Galicia Regional Government: “Consellería de cultura, educación e ordenación  
624 universitaria & GAIN, Xunta de Galicia, ED481B-2017/31”, Spain. This study was  
625 funded by IDEX grant BioDisphère from the University of Strasbourg and by the  
626 French National Research Agency ANR through grant ANR-18-CE04-0004-01, project  
627 DECISIVE. The authors wish to acknowledge two anonymous reviewers for helpful  
628 comments and suggestions, as well as Amélie Aubert, Boris Droz, Colin Fournier and  
629 Benoît Guyot for their help with analytical measurements.

630

## 631 **References**

- 632 Álvarez-Zaldívar P, Payraudeau S, Meite F, Masbou J, Imfeld G. Pesticide degradation  
633 and export losses at the catchment scale: Insights from compound-specific isotope  
634 analysis (CSIA). *Water Res* 2018; 139:198-207.
- 635 Babcsányi I, Chabaux F, Granet M, Meite F, Payraudeau S, Duplay J et al. Copper in  
636 soil fractions and runoff in a vineyard catchment: Insights from copper stable isotopes.  
637 *Sci Total Environ* 2016; 557-558:154-62.
- 638 Bai Z, Xu H-, He H-, Zheng L-, Zhang X-. Alterations of microbial populations and  
639 composition in the rhizosphere and bulk soil as affected by residual acetochlor. *Environ*  
640 *Sci Pollut Res* 2013; 20:369-79.
- 641 Baker KL, Marshall S, Nicol GW, Campbell CD, Nicollier G, Ricketts D et al.  
642 Degradation of metalaxyl-M in contrasting soils is influenced more by differences in  
643 physicochemical characteristics than in microbial community composition after re-  
644 inoculation of sterilised soils. *Soil Biol Biochem* 2010; 42:1123-31.

- 645 Bardgett RD, Van Der Putten WH. Belowground biodiversity and ecosystem  
646 functioning. *Nature* 2014; 515:505-11.
- 647 Bourceret A, Leyval C, De Fouquet C, Cébron A. Mapping the centimeter-scale spatial  
648 variability of PAHs and microbial populations in the rhizosphere of two plants. *PLoS*  
649 *ONE* 2015; 10.
- 650 Buerge IJ, Poiger T, Müller MD, Buser H-. Enantioselective degradation of metalaxyl  
651 in soils: Chiral preference changes with soil pH. *Environ Sci Technol* 2003; 37:2668-  
652 74.
- 653 Canarini A, Kaiser C, Merchant A, Richter A, Wanek W. Root exudation of primary  
654 metabolites: Mechanisms and their roles in plant responses to environmental stimuli.  
655 *Front Plant Sci* 2019; 10.
- 656 Chaudhry Q, Blom-Zandstra M, Gupta S, Joner EJ. Utilising the synergy between plants  
657 and rhizosphere microorganisms to enhance breakdown of organic pollutants in the  
658 environment. *Environ Sci Pollut Res* 2005; 12:34-48.
- 659 Chu B, Eivazi F. Enhanced dissipation of selected herbicides in a simulated organic  
660 matrix biobed: A system to control on-farm point-source pollution. *J Environ Qual*  
661 2018; 47:221-7.
- 662 Dennis PG, Miller AJ, Hirsch PR. Are root exudates more important than other sources  
663 of rhizodeposits in structuring rhizosphere bacterial communities? *FEMS Microbiol*  
664 *Ecol* 2010; 72:313-27.
- 665 Dhananjayan V, Jayakumar S, Ravichandran B. Conventional methods of pesticide  
666 application in agricultural field and fate of the pesticides in the environment and human  
667 health. *Controlled release of pesticides for sustainable agriculture* 2020; 1-39.
- 668 Diez MC, Elgueta S, Rubilar O, Tortella GR, Schalchli H, Bornhardt C et al. Pesticide  
669 dissipation and microbial community changes in a biopurification system: influence of  
670 the rhizosphere. *Biodegradation* 2017; 28:395-412.
- 671 Duplay J, Semhi K, Errais E, Imfeld G, Babcsanyi I, Perrone T. Copper, zinc, lead and  
672 cadmium bioavailability and retention in vineyard soils (Rouffach, France): the impact  
673 of cultural practices. *Geoderma* 2014; 230-231:318-28.
- 674 EFSA. Conclusion on the peer review of the pesticide risk assessment of the active  
675 substance metalaxyl-M. *EFSA J* 2015; 13.
- 676 Elsayed OF, Maillard E, Vuilleumier S, Nijenhuis I, Richnow HH, Imfeld G. Using  
677 compound-specific isotope analysis to assess the degradation of chloroacetanilide  
678 herbicides in lab-scale wetlands. *Chemosphere* 2014; 99:89-95.
- 679 Elsner M, Imfeld G. Compound-specific isotope analysis (CSIA) of micropollutants in  
680 the environment - current developments and future challenges. *Curr Opin Biotechnol*  
681 2016; 41:60-72.

- 682 Elsner M, Mckelvie J, Couloume GL, Lollar BS. Insight into methyl tert-butyl ether  
683 (MTBE) stable isotope fractionation from abiotic reference experiments. Environ Sci  
684 Technol 2007; 41:5693-700.
- 685 Elsner M, Zwank L, Hunkeler D, Schwarzenbach RP. A new concept linking  
686 observable stable isotope fractionation to transformation pathways of organic pollutants.  
687 Environ Sci Technol 2005; 39:6896-916.
- 688 EUROSTAT. Agri-environmental indicator - consumption of pesticides. 2020:1-11.  
689 [https://ec.europa.eu/eurostat/statistics-explained/index.php/Agri-](https://ec.europa.eu/eurostat/statistics-explained/index.php/Agri-environmental_indicator_-_consumption_of_pesticides)  
690 [environmental\\_indicator\\_-\\_consumption\\_of\\_pesticides](https://ec.europa.eu/eurostat/statistics-explained/index.php/Agri-environmental_indicator_-_consumption_of_pesticides)
- 691 EXTOWNET, The EXtension TOXicology NETwork, 2000. Pesticide information  
692 profile: metolachlor. Available at: extownet.orst.edu/.
- 693 Fernández-Calviño D, Cutillas-Barreiro L, Núñez-Delgado A, Fernández-Sanjurjo MJ,  
694 Álvarez-Rodríguez E, Nóvoa-Muñoz JC et al. Cu immobilization and *Lolium perenne*  
695 development in an acid vineyard soil amended with crushed mussel shell. Land Degrad  
696 Develop 2017; 28:762-72.
- 697 Fernández-Calviño D, Bååth E. Growth response of the bacterial community to pH in  
698 soils differing in pH. FEMS Microbiol Ecol 2010; 73:149-56.
- 699 Fernández-Calviño D, Bermúdez-Couso A, Nóvoa-Muñoz JC, Arias-Estévez M.  
700 Metalaxyl mobility in acid soils: evaluation using different methods. Int J Environ Sci  
701 Technol 2015; 12:2179-90.
- 702 Gangloff S, Stille P, Schmitt A, Chabaux F. Factors controlling the chemical  
703 composition of colloidal and dissolved fractions in soil solutions and the mobility of  
704 trace elements in soils. Geochim Cosmochim Acta 2016; 189:37-57.
- 705 Gevao B, Semple KT, Jones KC. Bound pesticide residues in soils: a review. Environ  
706 Pollut 2000; 108:3-14.
- 707 Giori FG, Tornisiello VL, Regitano JB. The role of sugarcane residues in the sorption  
708 and leaching of herbicides in two tropical soils. Water Air Soil Pollut 2014; 225.
- 709 Gluhar S, Kaurin A, Grubar T, Prosen H, Lestan D. Dissipation of mecoprop-P,  
710 isoproturon, bentazon and *S*-metolachlor in heavy metal contaminated acidic and  
711 calcareous soil before and after EDTA-based remediation. Chemosphere 2019;  
712 237:124513.
- 713 Hand LH, Gougoulis C, Bramke I, Thomas KA, Oliver RG. Evaluation of the  
714 rhizosphere contribution to the environmental fate of the herbicide prometryn. Environ  
715 Toxicol Chem 2020; 39:450-7.
- 716 Heyer R, Stan H-. Comparison of the leaching behaviour of alachlor and its metabolites  
717 under field and laboratory conditions. Int J Environ Anal Chem 1995; 58:173-83.

- 718 Hinsinger P, Bengough AG, Vetterlein D, Young IM. Rhizosphere: biophysics,  
719 biogeochemistry and ecological relevance. *Plant Soil* 2009; 321:117-52.
- 720 Hinsinger P, Gobran GR, Gregory PJ, Wenzel WW. Rhizosphere geometry and  
721 heterogeneity arising from root-mediated physical and chemical processes. *New Phytol*  
722 2005; 168:293-303.
- 723 Hinsinger P, Plassard C, Jaillard B. Rhizosphere: a new frontier for soil  
724 biogeochemistry. *J Geochem Explor* 2006; 88:210-3.
- 725 Huang H, Wang S, Lv J, Xu X, Zhang S. Influences of artificial root exudate  
726 components on the behaviors of BDE-28 and BDE-47 in soils: desorption, availability,  
727 and biodegradation. *Environ Sci Pollut Res* 2016; 23:7702-11.
- 728 Hunsche M, Damerow L, Schmitz-Eiberger M, Noga G. Mancozeb wash-off from apple  
729 seedlings by simulated rainfall as affected by drying time of fungicide deposit and rain  
730 characteristics. *Crop Prot* 2007; 26:768-74.
- 731 Imfeld G, Besaury L, Maucourt B, Donadello S, Baran N, Vuilleumier S. Toward  
732 integrative bacterial monitoring of metolachlor toxicity in groundwater. *Front Microbiol*  
733 2018; 9.
- 734 Ivdra N, Herrero-Martín S, Fischer A. Validation of user- and environmentally friendly  
735 extraction and clean-up methods for compound-specific stable carbon isotope analysis  
736 of organochlorine pesticides and their metabolites in soils. *J Chromatogr A* 2014;  
737 1355:36-45.
- 738 Jiang W, Gao J, Cheng Z, Wang P, Zhou Z, Liu D. The effect of antibiotics on the  
739 persistence of herbicides in soil under the combined pollution. *Chemosphere* 2018;  
740 204:303-9.
- 741 Jones DL, Nguyen C, Finlay RD. Carbon flow in the rhizosphere: carbon trading at the  
742 soil-root interface. *Plant Soil* 2009; 321:5-33.
- 743 Kuzyakov Y, Blagodatskaya E. Microbial hotspots and hot moments in soil: Concept &  
744 review. *Soil Biol Biochem* 2015; 83:184-99.
- 745 Kuzyakov Y, Razavi BS. Rhizosphere size and shape: temporal dynamics and spatial  
746 stationarity. *Soil Biol Biochem* 2019; 135:343-60.
- 747 Lefevre GH, Hozalski RM, Novak PJ. Root exudate enhanced contaminant desorption:  
748 an abiotic contribution to the rhizosphere effect. *Environ Sci Technol* 2013; 47:11545-  
749 53.
- 750 Lefrancq M, Jadas-Hécart A, La Jeunesse I, Landry D, Payraudeau S. High frequency  
751 monitoring of pesticides in runoff water to improve understanding of their transport and  
752 environmental impacts; 28242219. *Sci Total Environ* 2017; 587-588:75-86.
- 753 Masbou J, Drouin G, Payraudeau S, Imfeld G. Carbon and nitrogen stable isotope  
754 fractionation during abiotic hydrolysis of pesticides. *Chemosphere* 2018a; 213:368-76.

- 755 Masbou J, Meite F, Guyot B, Imfeld G. Enantiomer-specific stable carbon isotope  
756 analysis (ESIA) to evaluate degradation of the chiral fungicide Metalaxyl in soils. *J*  
757 *Hazard Mater* 2018b; 353:99-107.
- 758 Megharaj M, Ramakrishnan B, Venkateswarlu K, Sethunathan N, Naidu R.  
759 Bioremediation approaches for organic pollutants: a critical perspective. *Environ Int*  
760 2011; 37:1362-75.
- 761 Meite F, Alvarez-Zaldívar P, Crochet A, Wiegert C, Payraudeau S, Imfeld G. Impact of  
762 rainfall patterns and frequency on the export of pesticides and heavy-metals from  
763 agricultural soils. *Sci Total Environ* 2018; 616-617:500-9.
- 764 Melsbach A, Torrentó C, Ponsin V, Bolotin J, Lachat L, Prasuhn V et al. Dual-element  
765 isotope analysis of desphenylchloridazon to investigate its environmental fate in a  
766 systematic field study: a long-term lysimeter experiment. *Environ Sci Technol* 2020;  
767 54:3929-39.
- 768 Mommer L, Kirkegaard J, van Ruijven J. Root–root interactions: towards a rhizosphere  
769 framework. *Trends Plant Sci* 2016; 21:209-17.
- 770 Pérez-Rodríguez P, Paradelo M, Soto-Gómez D, Fernández-Calviño D, López-Periago  
771 JE. Modeling losses of copper-based fungicide foliar sprays in wash-off under  
772 simulated rain. *Int J Environ Sci Te* 2015; 12:661-72.
- 773 Pérez-Rodríguez P, Soto-Gómez D, De La Calle I, López-Periago JE, Paradelo M.  
774 Rainfall-induced removal of copper-based spray residues from vines. *Ecotoxicol*  
775 *Environ Saf* 2016; 132:304-10.
- 776 Ponsin V, Torrentó C, Lihl C, Elsner M, and Hunkeler D. Compound-Specific Chlorine  
777 Isotope Analysis of the Herbicides Atrazine, Acetochlor, and Metolachlor. *Anal Chem*  
778 2019; 91(22):14290–14298
- 779 Qian Y, Chen K, Liu Y, Li J. Assessment of hexachlorocyclohexane biodegradation in  
780 contaminated soil by compound-specific stable isotope analysis. *Environ Pollut* 2019;  
781 254:113008.
- 782 Ren X, Zeng G, Tang L, Wang J, Wan J, Liu Y et al. Sorption, transport and  
783 biodegradation – an insight into bioavailability of persistent organic pollutants in soil.  
784 *Sci Total Environ* 2018; 610-611:1154-63.
- 785 Rousk J, Brookes PC, Bååth E. Investigating the mechanisms for the opposing pH  
786 relationships of fungal and bacterial growth in soil. *Soil Biol Biochem* 2010; 42:926-34.
- 787 Sakaliene O, Papiernik SK, Koskinen WC, Spokas KA. Sorption and predicted mobility  
788 of herbicides in Baltic soils. *J Environ Sci Health Part B Pestic Food Contamin Agric*  
789 *Wastes* 2007; 42:641-7.
- 790 Sukul P, Spiteller M. Influence of biotic and abiotic factors on dissipating metalaxyl in  
791 soil. *Chemosphere* 2001; 45:941-7.

- 792 Thornthwaite, C.W., and Mather, J.R. The water balance. Laboratory of Climatology,  
793 Publications in Climatology ed. v. 8, no. 1, p. 1–104.; 1955.
- 794 Torabi E, Wiegert C, Guyot B, Vuilleumier S, Imfeld G. Dissipation of *S*-metolachlor  
795 and butachlor in agricultural soils and responses of bacterial communities: insights from  
796 compound-specific isotope and biomolecular analyses. *J Environ Sci* 2020; 92:163-75.
- 797 University of Hertfordshire. IUPAC: Alachlor 2019.
- 798 Violante A, Caporale AG. Biogeochemical processes at soil-root interface. *J Soil Sci*  
799 *Plant Nutri* 2015; 15:422-48.
- 800 Wang G, Liu Y, Tao W, Zhao X, Wang H, Lou Y et al. Assessing microbial  
801 degradation degree and bioavailability of BDE-153 in natural wetland soils: implication  
802 by compound-specific stable isotope analysis. *Environ Pollut* 2020; 260:114014.
- 803 Xu Z, Liu W, Yang F. A new approach to estimate bioavailability of pyrethroids in soil  
804 by compound-specific stable isotope analysis. *J Hazard Mater* 2018; 349:1-9.
- 805 Yu Q, Zhang P, He Y, Xu Z, He X, Hu Y et al. Dissipation dynamics and residue of  
806 four herbicides in paddy fields using HPLC-MS/MS and GC-MS. *Int J Environ Res*  
807 *Public Health* 2019; 16.
- 808 Zhang R, Vivanco JM, Shen Q. The unseen rhizosphere root–soil–microbe interactions  
809 for crop production. *Curr Opin Microbiol* 2017; 37:8-14.
- 810 Zheng HH, Ye CM. Identification of UV photoproducts and hydrolysis products of  
811 butachlor by mass spectrometry. *Environ Sci Technol* 2001; 35, 2889e2895.