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

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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 γ -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*₁₁ (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 m² 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 m²). 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 μm) 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 g/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 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[®] 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 ± 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 (μg) in leachates were estimated from individual pesticide
 251 concentrations 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 [μg] 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 ≥ 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 ε relates changes of isotope ratios to the extent
 300 of transformation. The ε_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 ε_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 (ε_c) of anilide pesticides retrieved from Elsayed (2014) for ACE (-
 315 3.5 ± 0.5) and ALA (-2.0 ± 0.3), Masbou et al., (2018a, 2018b) for BUT (-3.7 ± 0.7) and
 316 MTY (-2.0 ± 1.3 in F and -0.9 ± 0.5 in V soil), respectively and Álvarez-Zaldívar et al.
 317 (2018) for SM (-1.5 ± 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)/cm³ 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⁻¹) than in the forest (0.01 d⁻¹) 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 ϵ_{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 ϵ_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 ϵ_C obtained previously in
521 Elsayed et al., (2014) in a wetland mesocosms and significantly higher than the values
522 of ϵ_C obtained during abiotic hydrolysis by Masbou et al., (2018a). ϵ_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 ± 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 Λ values
560 from the slopes (SI-O). Distinct Λ 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 Λ value retrieved from the planted
563 mesocosm was like the Λ 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

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630

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