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1 **Fate of Sulfamethoxazole, its main metabolite N-ac-Sulfamethoxazole and Ciprofloxacin**  
2 **in agricultural soils amended or not by organic waste products**

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11

12 **Abstract:**

13 Spreading organic waste products (OWP) issued from sewage sludge or manures into soil  
14 may disseminate antibiotics with unknown risks for human health and environment. Our  
15 objectives were to compare the fate of two sulfonamides, sulfamethoxazole (SMX) and its  
16 metabolite N-acetyl-sulfamethoxazole (N-ac-SMX), and one fluoroquinolone, ciprofloxacin  
17 (CIP), in an unamended soil, and two soils regularly amended since 1998 with a sewage  
18 sludge and green waste compost and with farmyard manure respectively. Incubations of soil  
19 spiked with <sup>14</sup>C labelled SMX or N-ac-SMX (0.02 mg.kg<sup>-1</sup>) or CIP (0.15 mg.kg<sup>-1</sup>) allowed a  
20 quantification of radiolabeled molecules in the mineralized, easily, hardly and non-extractable  
21 fractions after 3 and 156 days. Nature of <sup>14</sup>C molecules was also analyzed by HPLC in  
22 extractable fractions after 3 and 156 days.

23 SMX and N-ac-SMX dissipation was fast and due to i) mineralization (~10% of recovered <sup>14</sup>C  
24 after 156 days) or incomplete degradation (production of metabolites), ii) adsorption, even if

25 both sulfonamides present low  $K_d$  ( $<3 \text{ L.kg}^{-1}$ ) and iii) formation of non-extractable residues  
26 (NER), representing more than 50% of recovered radioactivity.

27 N-ac-SMX was more mineralized than SMX, and formed more progressively NER, after a  
28 step of deacetylation. Adsorption of CIP was fast and formed mainly NER ( $> 72\%$ ) whereas  
29 its mineralization was negligible. Repeated applications of OWP tend to enhance adsorption  
30 of antibiotics and lower their degradation, through the quantity and quality of the built up soil  
31 organic matter. If applications of sewage sludge compost favor adsorption and inhibit  
32 mineralization, applications of manure boost the formation of non-extractable residues.

33

34 **Keywords:** Soil; Antibiotic; Metabolite; Organic waste products; Biodegradation; Adsorption

35

36 **Highlights:**

37 N-ac-SMX slightly more mobile and more mineralized than SMX during soil incubations

38 Differences in fates of N-ac-SMX and SMX partly due to a step of deacetylation

39 SMX more available in soil than CIP but both mainly non-extractable after 156 days

40 Regular organic amendments enhance sorption and lower mineralization of antibiotics

41

42

## 43 **1. Introduction**

44 The increasing occurrence of antibiotics (ATB) in soils, surface water and ground water  
45 becomes of more and more concern because of environmental and human health threat  
46 (Boxall et al., 2012).

47 Once in the soil, ATB may be degraded by biotic or abiotic path, retained on soil constituents  
48 and/or transferred to surface and groundwater through runoff and infiltration (Jechalke et al.,  
49 2014). Volatilization and photodegradation of antibiotics are generally negligible processes in  
50 soils (Ozaki et al., 2011; Jechalke et al., 2014). Indeed, the fate of antibiotics depends on  
51 antibiotics and soil properties. Generally detected in the  $\mu\text{g}$ - $\text{mg}$  per kg range (Golet et al.,  
52 2002; Boxall et al., 2004), ATB may persist for years in soils (Du and Liu, 2012) while  
53 maintaining an activity even at a low dose (Boxall et al., 2004).

54 One of the entry routes of veterinary and human antibiotics into agricultural soils is the  
55 application of organic waste products (OWP) (Kümmerer, 2001), which is a worldwide  
56 increasingly common practice of recycling valuable nutrients and organic matter (Chang et  
57 al., 1995). However OWP may contain antibiotics. When administered to livestock, 10 to  
58 90% of ATB are excreted as parent compounds or metabolites (Sarmah et al., 2006).  
59 Consequently ATB and metabolites can be present directly in livestock effluents (Boxall et  
60 al., 2004). ATB in human's urine and feces are not completely eliminated in waste water  
61 plants and can persist in effluents and/or sludge (Nieto et al., 2010).

62 OWP addition may also impact the fate of antibiotics in soils, through changing soil physical,  
63 chemical and biological properties (Li et al., 2015). Contradictory results were obtained under  
64 laboratory conditions. Some works highlighted a stimulation of ATB biodegradation after  
65 OWP addition (Accinelli et al., 2007; Zhang et al., 2017) while others showed an increase of  
66 ATB adsorption and inhibition of mineralization (Li et al., 2015).

67 However studies based on addition of OWP into soils in the lab miss the long-term effects of  
68 OWP applications on soil and their indirect effect on the fate of ATB. In this study, we  
69 compared the fate of ATB in three soils from the same field experiment. Two have been  
70 amended every two years since 1998, with a farmyard manure (FYM) or a co-compost of  
71 sewage sludge and green waste (SGW), and the third one has never been amended  
72 (CONT). We focused on two ATB widely used in human and veterinary medicine,  
73 sulfamethoxazole (SMX) and ciprofloxacin (CIP), belonging respectively to sulfonamides and  
74 fluoroquinolones families. These molecules are among the most frequently detected ATB in  
75 the environment (Barnes et al., 2004; Kolpin et al., 2002), and present contrasting behavior in  
76 soils. SMX is relatively mobile and rapidly degraded with half-lives ranging from a few hours  
77 to a few days (Salvia et al., 2014; Srinivasan and Sarmah, 2014), while CIP is known to have  
78 a high affinity to soils (Nowara et al., 1997; Vasudevan et al., 2009) and may persist for  
79 several months to years (Hamscher et al., 2005; Rosendahl et al., 2012). However some  
80 studies suggested that sulfonamides may be more persistent than would be predicted from  
81 laboratory controlled studies (Bialk et al., 2005). The different characteristics of CIP and  
82 SMX make them good candidates to represent a range of antibiotics in the environment (Chen  
83 et al., 2011).

84 We also considered the main metabolite of SMX, the N-acetyl-sulfamethoxazole (N-ac-  
85 SMX). When excreted, this metabolite accounts for approximately 50% of the administered  
86 SMX. But N-ac-SMX may undergo deacetylation in the environment and SMX can be  
87 regenerated (Göbel et al., 2005).

88 Thus the objectives of this study were to determine i) the fate of antibiotics with contrasted  
89 characteristics in soils and ii) how long-term applications of different OWP to soils impacted  
90 the fate of these antibiotics.

91 We monitored  $^{14}\text{C}$ -labelled antibiotics during 6 months incubations by quantifying  
92 radioactivity in the mineralized, extractable and non-extractable fractions, and by qualifying  
93 radioactivity (parent molecules or metabolites) in the extractable fractions.

94

## 95 **2. Materials and methods**

### 96 *2.1. Antibiotics*

97  $^{14}\text{C}$ -ring-labeled sulfamethoxazole (chemical purity 98%, specific activity 77  $\text{mCi}\cdot\text{mmol}^{-1}$ )  
98 and  $^{14}\text{C}$ -ring-labeled N-acetyl-sulfamethoxazole (chemical purity 93%, specific activity 77  
99  $\text{mCi}\cdot\text{mmol}^{-1}$ ) were purchased from Isobio (ARC).  $^{14}\text{C}$ -ring-labeled ciprofloxacin (chemical  
100 purity 98.4%, specific activity 15  $\text{mCi}\cdot\text{mmol}^{-1}$ ), was purchased from Moravek Biochemical.  
101 The structures, pKa, and labelling of molecules are presented in Fig. S1.

102

### 103 *2.2. Soil sampling*

104 Soils were sampled in 2014 from the ploughed layers (0-25 cm) of the experimental field  
105 QualiAgro (48°53' N, 1°58' E) located at Feucherolles (Yvelines, France): a soil control that  
106 has never received OWP and 2 soils amended every 2 years since 1998 with a co-compost of  
107 green waste and sewage sludge (SGW) or farmyard manure (FYM) (Houot et al., 2002). At  
108 sampling, amended soils (SGW or FYM) have received 9 applications of OWP. The soil is a  
109 silt loam Glossic Luvisol and contains on average 19% clay, 75% silt and 6% sand in the  
110 ploughed layer. Clay minerals identified are kaolinite, illite and smectite, with traces of  
111 chlorite (Cornu, personal communication). The field is cropped with a biannual rotation  
112 winter wheat-maize. Selected properties of the soil samples and of the OWP applied in 2013  
113 are listed in Table 1. The soils were homogenized, sieved through a 4-mm mesh and stored 3  
114 weeks at 4°C in the dark before using.

115

116 **Table 1**

117 Physicochemical properties of soils (CONT, SGW, FYM) and OWP used as amendment in  
 118 2013

	Soils			OWP	
	CONT	SGW	FYM	Compost sewage sludge-green waste	Farmyard manure
Organic carbon (g.kg <sup>-1</sup> dw)	10.2	17.6	15.5	257.3	354.7
Total nitrogen (g.kg <sup>-1</sup> dw)	1.0	1.7	1.4	25.5	20.1
pH	6.7	6.8	7.3	7.7	8.8
CEC (cmol+.kg <sup>-1</sup> )	7.6	9.8	9.9	-	-
C/N	10.2	10.4	11.1	9.9	17.6
I <sub>ROC</sub> <sup>a</sup>	-	-	-	78	67
[SMX] (µg.kg <sup>-1</sup> soil dw) <sup>b</sup>	nd	nd	nd	nd	nd
[CIP] (µg.kg <sup>-1</sup> soil dw) <sup>b</sup>	nd	7.3 ± 1.9	nd	309 ± 22	nd

119 <sup>a</sup> Proportion of total organic carbon potentially incorporated into soil organic matter  
 120 (Lashermes et al., 2009).

121 <sup>b</sup> Concentrations of ATB were analysed in OWP of 2013 and soil sampled in November 2104  
 122 (Bourdat-Deschamps et al., 2017).

123 nd: not detected

125 *2.3. Sorption Coefficient of SMX and N-ac-SMX*

126 Sorption coefficients (K<sub>d</sub>) of SMX and N-ac-SMX were determined on gamma-ray sterilized  
 127 (40–70 kGy) samples of the 3 studied soils as follows: three replicates of 3 g equivalent dry  
 128 soil were mixed with 10 ml of CaCl<sub>2</sub> 0.01 M solution sterilized by filtration at 0.2 µm and  
 129 7.96 µl of <sup>14</sup>C labelled SMX or 10.14 µl of <sup>14</sup>C labelled N-ac-SMX (0.02 µg.g<sup>-1</sup> soil), before  
 130 shaking at 15 rotations.min<sup>-1</sup> for 24 h in the dark at 20°C. Samples were then centrifuged 10  
 131 min at 34155 g at 20°C and the <sup>14</sup>C remaining in the supernatants was quantified by liquid  
 132 scintillation counting.

133 Then K<sub>d</sub> (L.kg<sup>-1</sup>) was calculated as follows:

134 
$$K_d = C_{ads}/C_e$$

135 Cads was the concentration of ATB adsorbed on soil ( $\text{mg.kg}^{-1}$ ) and Ce, the concentration of  
136 ATB in soil solution after equilibrium ( $\text{mg.L}^{-1}$ ).

137

#### 138 2.4. *Incubations*

139 Ten grams (equivalent dry soil) of CONT, SGW and FYM soils were amended with 1 ml of  
140 solutions of  $^{14}\text{C}$ -SMX,  $^{14}\text{C}$ -N-ac-SMX or  $^{14}\text{C}$ -CIP to provide final concentrations of 0.02, 0.02  
141 or  $0.15 \mu\text{g.g}^{-1}$  soil respectively ( $1543 \text{ MBq.mM}^{-1}$  per sample) and a moisture content  
142 corresponding to 80% of maximum water holding capacity (0.22 g of water per g of soil). Six  
143 replicates per treatment were prepared. Spiked soils were incubated in 1 L airtight jars in  
144 presence of 2 vials containing respectively 10 ml of deionized water and 6 ml of sodium  
145 hydroxide 1N, at  $28^\circ\text{C}$  for 156 days.

146 At 3 days and then weekly,  $^{14}\text{C}$ -activity in the NaOH solutions was measured with a TriCarb  
147 liquid scintillation counter after mixing with 10 mL of UltimaGold liquid scintillation  
148 cocktail. Samples were kept for at least 24 h in the dark before counting to avoid potential  
149 chemiluminescence. The counting time was 10 min.

150 The opening of the jars to replace the NaOH traps ensured aerobic conditions during all  
151 incubation time. The soil moisture was checked over time and readjusted when necessary to  
152 80% of WHC by adding deionized water.

153

#### 154 2.5. *Quantification of $^{14}\text{C}$ residues*

155 At days 3 and 156, three successive extractions were performed to quantify the distribution of  
156 radioactivity from  $^{14}\text{C}$ -SMX,  $^{14}\text{C}$ -N-ac-SMX and  $^{14}\text{C}$ -CIP in the easily and hardly extractable  
157 and the non-extractable fractions. The extraction protocol was adapted from Ferhi et al.  
158 (2016).



159 To measure the easily extractable fractions, 30 ml of cyclodextrin HPCD (0.05 mol.L<sup>-1</sup>) were  
160 added to soil samples in 250 ml PPCO tubes. After shaking during 16 hours at 15  
161 rotations.min<sup>-1</sup>, samples were centrifuged (20 min, 20°C, 34155 g). Supernatants were  
162 weighted and mixed with 10 ml of Ultima Gold XR scintillation liquid (Perkin Elmer) for  
163 counting. The next extraction was performed by adding acetonitrile MeCN (99.9%  
164 Acetonitrile Carlo Erba Reagents Dasit Group, 27.5 ml) combined with a Mc Ilvaine pH9  
165 buffer solution (9.3 mL of 1/29 (v/v) citric acid/phosphate). pH9 was reached with addition of  
166 NaOH 10 N. Samples were ultra-sonicated twice 10 min before centrifugation (20 min, 20°C,  
167 34155 g). Radioactivity was quantified in the supernatants by liquid scintillation. The last  
168 extraction was performed by addition of MeCN (99.9% Acetonitrile Carlo Erba Reagents  
169 Dasit Group, 27.5 ml) combined with a Mc Ilvaine pH2 buffer solution (11 mL of 27/3 (v/v)  
170 citric acid/phosphate). pH2 was reached by adding HCl 37%. Samples were ultra-sonicated  
171 twice for 10 min, before centrifugation (20 min, 20°C, 34155 g). Because of a salting out  
172 effect due to the high quantity of EDTA salt, two phases were obtained. Radioactivity was  
173 quantified in the lower and upper phases by liquid scintillation. The hardly extractable  
174 fraction was defined as the sum of pH9 and pH2 extracts.

175 After extractions, soil samples containing non-extractable <sup>14</sup>C-residues (NER) were air dried  
176 at 35°C. Radioactivity from NER was measured by combustion (Biological Oxidizer OX 700,  
177 Zinsser Analytic, Frankfurt, Germany) and trapping of the <sup>14</sup>CO<sub>2</sub> in Carbosorb and liquid  
178 scintillation counting (Packard Instruments, Meriden, CT, USA).

179

## 180 2.6. *Qualitative analysis of <sup>14</sup>C residues in extractable fractions*

181 A qualitative analysis of <sup>14</sup>C in HPCD and MeCN extracts was carried out by high-  
182 performance liquid chromatography (HPLC) with a Waters (Milford,MA, USA)  
183 chromatography appliance (Systemcontroller 610, Autosampler 717) coupled with a

184 radioactive flow detector (Flo-one A-500, Packard–Radiomatic) when radioactivity in  
185 samples was greater than 250 Bq.mL<sup>-1</sup>. In certain cases, the 3 repetitions per extracts were  
186 pooled to obtain sufficient radioactivity for analysis (Table S1). HPCD extracts were  
187 concentrated on SPE cartridges (Strata X-A 33 µm Polymeric Stron Anion, Phenomenex) and  
188 then with nitrogen before HPLC analyses. Radioactivity in HPCD extracts was too low after  
189 156 days of incubation to be analyzed. MeCN-McIlvaine extracts were evaporated before  
190 addition of 2 mL methanol. After centrifugation, samples were analyzed by HPLC.  
191 Radioactivity in the lower phase after MeCN-McIlvaine extraction was insufficient to be  
192 analyzed. Samples analyzed by HPLC (Perkin Elmer: Flexar System Quaternary Pump,  
193 Automatic Sample, PDA detector, Chromera® CDS piloting and acquisition Software;  
194 Column: Waters X-Bridge C18 5µ / 4.6×250 mm; detector: Radio-HPLC FLO  
195 ONE/Beta/Gamma C150TR; elution method: ACN + 0.01% TFA and H<sub>2</sub>O + 0.01% TFA) are  
196 synthetized in Table S1.

197

## 198 2.7. Statistical analyses

199 Statistical analyses, using R software, were performed *via* ANOVA and Tukey tests when  
200 values were parametric, Kruskal and Dunn tests when values were non-parametric, to  
201 compare the fate of <sup>14</sup>C SMX, N-ac-SMX and CIP in the 3 studied soils at d3 and d156. P-  
202 values less than 0.05 were considered as statistically significant.

203

204

205 **3. Results**

206 *3.1. Fate of SMX and N-ac-SMX into soils amended or not with OWP*

207 *3.1.1. Kd of SMX and N-ac-SMX*

208 Kd of SMX was lower in CONT than in SGW and intermediate in FYM (Table 2). Kd of N-  
209 ac-SMX was significantly different in the three soils, in the following order:  
210 SGW>CONT>FYM (Table 2).

211

212 **Table 2**

213 Sorption coefficients (Kd) of SMX and N-ac-SMX (L.kg<sup>-1</sup>) added at a concentration of 0.02  
214 µg.g<sup>-1</sup> soil in sterilized CONT, SGW and FYM soils (mean ± standard-deviation from 3  
215 replicates). Different letters indicate statistical differences (P<0.05) between the three soils.

<b>Kd (L.kg<sup>-1</sup>)</b>	<b>SMX</b>		<b>N-ac-SMX</b>		<b>SMX/N-ac-SMX</b>
CONT	2.58 ± 0.11	b	1.21 ± 0.03	b	2.1
SGW	3.20 ± 0.10	a	1.33 ± 0.00	a	2.4
FYM	2.95 ± 0.07	ab	1.02 ± 0.02	c	2.9

216

217 Kd of SMX appeared highly correlated to the C content of soils (R<sup>2</sup>=0.94) in contrast to N-ac-  
218 SMX (R<sup>2</sup>=0.008).

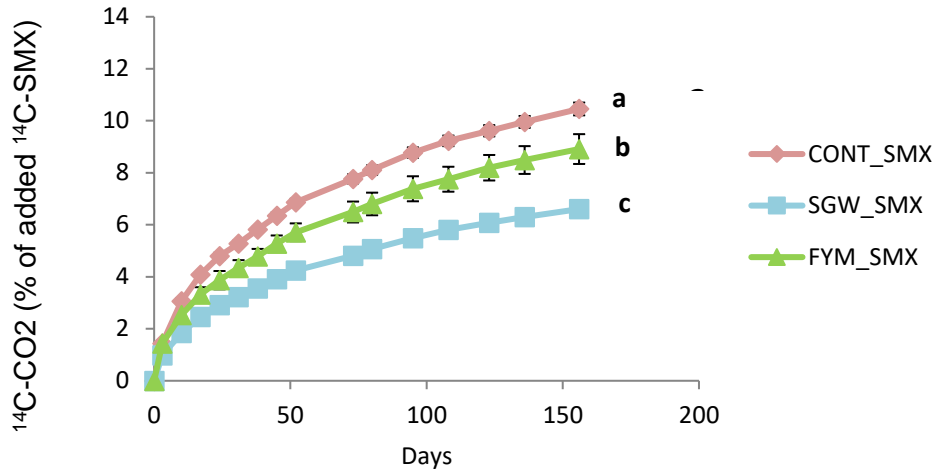
219 In the three soils, Kd of SMX was two to three folds higher than Kd of N-ac-SMX. In all  
220 cases, at a concentration of 0.02 µg.g<sup>-1</sup>, Kd values were low, whatever the molecules and  
221 whatever the soils.

222

223 *3.1.2. Fate of SMX*

224 SMX mineralization started without latency in the 3 soils and reached 6.6, 8.9 and 10.5% of  
225 added <sup>14</sup>C-SMX in the SGW, FYM and CONT soils respectively after 156 days (Fig. 1).

226 Although low in all cases, SMX mineralization was significantly greater in CONT than in  
227 FYM and SGW.



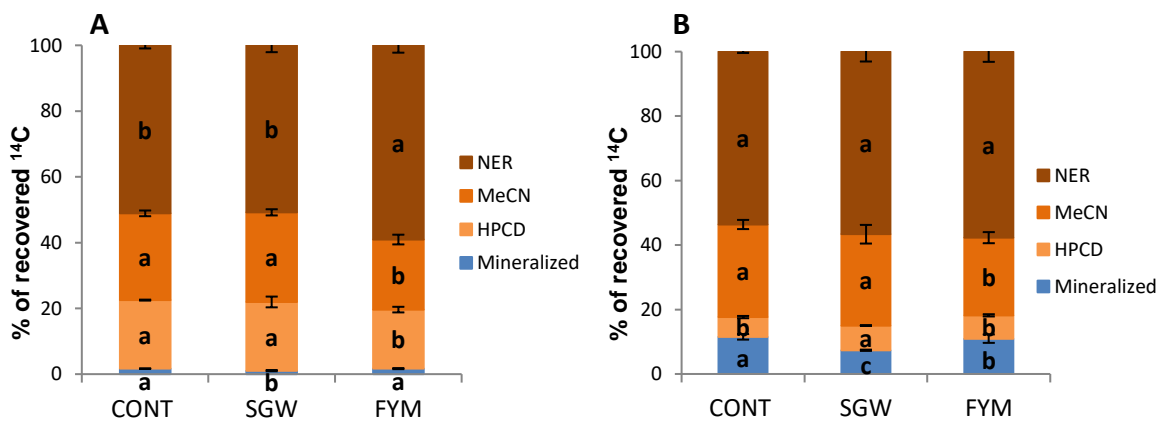
228

229 **Fig. 1.** Cumulative <sup>14</sup>C-SMX mineralization in the control soil and in the soils that have  
 230 received 9 applications of a co-compost of sewage sludge and green waste (SGW) or  
 231 farmyard manure (FYM). Error bars indicate standard deviations of 3 replicates.

232

233 The total recovery of <sup>14</sup>C in the mineralized, extractable (HPCD and MeCN) and non-  
 234 extractable <sup>14</sup>C fractions reached 87.4-88.8% of added <sup>14</sup>C-SMX after 3 days and 81.7-91%  
 235 after 156 days. For interpretation, the results were expressed as percentages of recovered <sup>14</sup>C.  
 236 The sum of all fractions corresponds therefore to 100% (Fig. 2).

237



238

239 **Fig. 2.** Distribution of  $^{14}\text{C}$ -SMX in the different soil fractions (mineralized, HPCD and MeCN  
240 extractable fractions, non-extractable residues), at (A) day 3, and (B) day 156, in the control  
241 soil (CONT) and the soils amended with a sewage sludge-green waste compost (SGW) or  
242 farmyard manure (FYM).

243  
244 At d3 (Fig. 2A),  $^{14}\text{C}$  was mainly distributed in the extractable and non-extractable fractions.  
245 SMX mineralization was negligible and varied between 1.1% (FYM) and 1.7% (CONT and  
246 SGW). Radioactivity in easily extractable (HPCD) fraction ranged between 17.9% (FYM) and  
247 20.9% (SGW) of recovered  $^{14}\text{C}$ . In hardly extractable (MeCN) fraction,  $^{14}\text{C}$  ranged between  
248 21.3% (FYM) and 27.3% (SGW). HPCD and MeCN extractable fractions were significantly  
249 lower in FYM. NER represented more than half of the recovered radioactivity as early as 3  
250 days (Fig. 2A). They were significantly higher in FYM than in CONT and SGW.

251 Between 3 and 156 days, we observed mainly a decrease of the HPCD fraction (from about  
252 20% to 6.1-7.7%) and an increase of the mineralized fraction. During the same period,  
253 mineralization was multiplied by a factor of about 6.5 and reached 7.4% (SGW), 11.0%  
254 (FYM) and 11.5% (CONT) of recovered  $^{14}\text{C}$  (Fig. 2B).

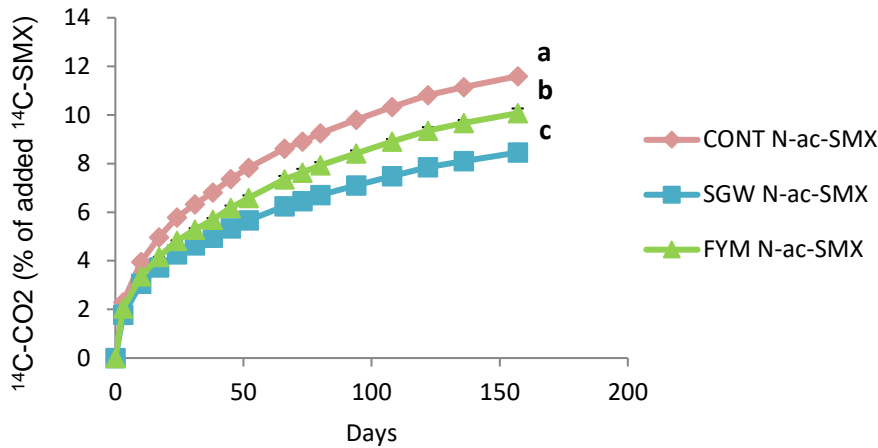
255 The MeCN fraction slightly but significantly increased to reach 24.1-28.7%, whereas NER  
256 did not significantly vary between d3 and d156.

257 After 156 days, CONT presented the highest SMX mineralization and SGW the lowest  
258 mineralization and the highest HPCD extractable fraction. At last, FYM presented the lowest  
259 MeCN  $^{14}\text{C}$  fraction.

260

261 3.1.3. Fate of N-ac-SMX

262 As for SMX, N-ac-SMX was immediately mineralized and cumulative <sup>14</sup>C-CO<sub>2</sub> ranged  
 263 between 8.5 and 11.6% at d156 (Fig. 3). As for SMX, N-ac-SMX mineralization was higher  
 264 in CONT than in amended soils and higher in FYM compared to SGW.

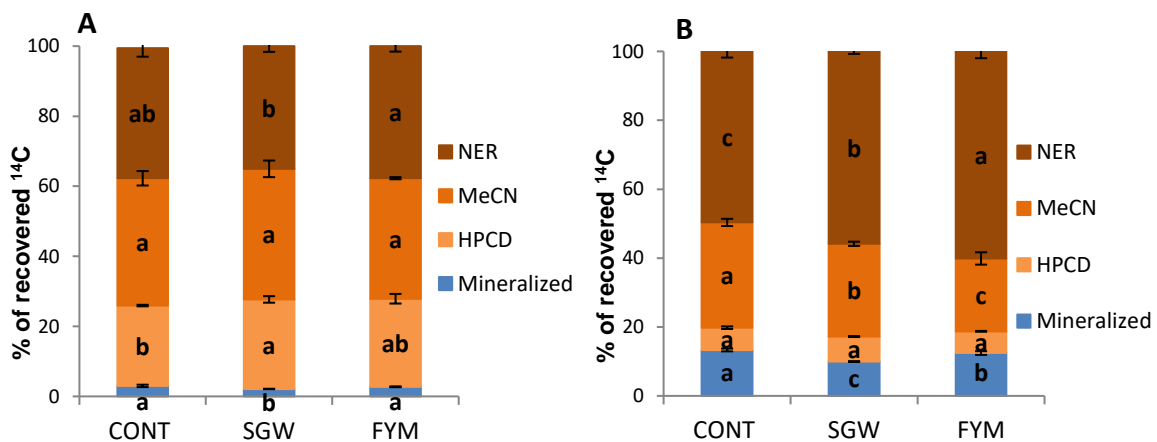


265

266 **Fig. 3.** Cumulative <sup>14</sup>C N-ac-SMX mineralization in the control soil (CONT) and the soils  
 267 amended with a sewage sludge-green waste compost (SGW) or farmyard manure (FYM).  
 268 Error bars indicate standard deviations of 3 replicates.

269

270 The total recovery of <sup>14</sup>C in the different fractions ranged from 71.7 to 82.2% of added <sup>14</sup>C N-  
 271 ac-SMX after 3 days and from 82.1 to 86.8% after 156 days. For interpretation, the results  
 272 were expressed as percentages of recovered <sup>14</sup>C (Fig. 4).



273

274 **Fig. 4.** Distribution of  $^{14}\text{C}$ -N-ac-SMX in the different soil fractions (mineralized, HPCD and  
275 MeCN extractable fractions, non-extractable residues), at (A) day 3, and (B) day 156, in the  
276 control soil (CONT) and the soils amended with a sewage sludge-green waste compost  
277 (SGW) or farmyard manure (FYM).

278  
279 After 3 days, N-ac-SMX mineralization was low (2.1-3.0%) and followed the order  
280  $\text{CONT} \geq \text{FYM} > \text{SGW}$ . The HPCD fractions represented 22.9-25.6% of recovered  $^{14}\text{C}$  and were  
281 significantly higher in SGW than in CONT, FYM presenting intermediate values. Hardly  
282 (MeCN) extractable fractions were not significantly different in the 3 soils (34.4-37.3%).  
283 NER varied between 35% (SGW) and 37.7% (FYM).

284 Between 3 and 156 days, mineralization increased by a 4.4-4.8 factor. NER increased to reach  
285 49.7% (CONT), 55.9% (SGW) and 60.1% (FYM) of recovered  $^{14}\text{C}$ . On the contrary, the  
286 HPCD and MeCN  $^{14}\text{C}$  fractions decreased and ranged between 6.3-7.3% and 21.2-30.6%  
287 respectively. At d156, the HPCD fractions were not significantly different between the 3 soils.  
288 CONT presented the highest mineralized and MeCN fractions. FYM was characterized by a  
289 lower MeCN fraction and higher NER. The majority of  $^{14}\text{C}$  was concentrated in the NER for  
290 the 3 soils.

291

#### 292 3.1.4. Comparison between SMX and N-ac-SMX at d3 and d156

293 At d3, radioactivity in the mineralized, HPCD and MeCN extractable fractions was  
294 significantly lower for SMX than for N-ac-SMX (Table S2). The mineralization from  $^{14}\text{C}$ -  
295 SMX was about half of that of N-ac-SMX. So, at d3, N-Ac-SMX was more mineralized and  
296 remained more extractable than SMX. At the opposite, addition of SMX produced more NER  
297 than N-ac-SMX. After 156 days, the HPCD fraction was significantly higher for SMX than  
298 N-ac-SMX except in CONT where it was similar. The MeCN fraction was similar for SMX

299 and N-ac-SMX in the three soils. NER were significantly higher in CONT after addition of  
300 SMX while they were similar for SMX and N-ac-SMX in SGW and FYM. When comparing  
301 fraction by fraction, differences between SMX and N-ac-SMX were similar for the 3 soils.

302

### 303 3.1.5. Qualitative analysis of $^{14}\text{C}$ in extractable fractions

304 Three days after SMX addition, HPCD fractions that represented around 20% of recovered  
305  $^{14}\text{C}$ , were mainly composed of  $^{14}\text{C}$ -SMX (71.3-80.5% of extractable  $^{14}\text{C}$ ). Four other  
306 metabolites including N-ac-SMX were detected but in smaller quantities. Metabolites differed  
307 among the 3 soils (Table 3).

308 In the MeCN pH9 fraction, we quantified no SMX but polar metabolites (M1, M2, M3) and  
309 an apolar metabolite (M9) at d3. Results were similar for the 3 soils. Between 3 and 156 days,  
310 metabolites evolved differently in the MeCN pH9 extracts. M2 and M3 disappeared while M9  
311 and especially M1 increased. M1 (66.7-83.4% of extractable  $^{14}\text{C}$  and 10.1-11.9% of total  $^{14}\text{C}$ ),  
312 the main metabolite and M9 were present in the 3 soils. M5 seemed specifically appear in the  
313 amended soils after 156 days.

314 SMX was present in the upper phase of MeCN pH2 extracts and represented 46.1 to 100% of  
315 extractable  $^{14}\text{C}$  (3.9 and 7.7% of total  $^{14}\text{C}$ ) in CONT and SGW respectively. SMX was  
316 predominant in this fraction for the 3 soils but metabolites (M6, M8, M9) were detected at  
317 smaller doses in one or another soil. CONT contained a greater diversity of metabolites than  
318 amended soils. In the MeCN pH2 extracts, SMX decreased over time but was still present at  
319 d156 in CONT and SGW. M8 and M9 disappeared and M4 and M6 remained the only  
320 quantifiable metabolites, representing 22.8-66% of extractable  $^{14}\text{C}$  (2.4-7% of total  $^{14}\text{C}$ ).

321 Three days after its addition, N-ac-SMX has almost completely disappeared in the HPCD  
322 fraction, subsisting only in CONT at about 12% of recovered  $^{14}\text{C}$ , and was converted into  
323 SMX and other metabolites (M6 et M7). SMX was present in the 3 soils but in larger



324 quantities in the amended soils compared to CONT. CONT also presented a higher diversity  
325 of metabolites.

326 The metabolites present in the MeCN pH9 fraction 3 days after N-ac-SMX addition were the  
327 same in the 3 soils (M1, M2, M3, M9) with similar distributions to those obtained after SMX  
328 addition.

329 In the MeCN pH2 extracts at d3, N-ac-SMX subsisted around 12% of extractable  $^{14}\text{C}$  in the 3  
330 soils and was mostly transformed into SMX (70%-87%).

331 At d156, only the MeCN pH9 extracts contained a sufficient radioactivity and could be  
332 analyzed by HPLC. Between d3 and d156, the polar metabolite M1 increased by a factor of  
333 2.3 to 3.5 and represented 59-71% of extractable  $^{14}\text{C}$  in the 3 soils. M2 and M3 decreased or  
334 disappeared and M9 increased to reach around 20% of extractable  $^{14}\text{C}$  in CONT and SGW. As  
335 for SMX, M5 appeared only in amended soils. The evolution of the MeCN pH9 metabolites  
336 was the same for SMX and N-ac-SMX (Table 3).

337

338 **Table 3**

339 Qualitative analysis of  $^{14}\text{C}$  by HPLC after addition of SMX or N-ac-SMX in the easily extractable fraction (HPCD), hardly extractable fraction at  
 340 pH9 (MeCN pH9) and hardly extractable fraction (upper phase) at pH2 (MeCN pH2) of CONT, SGW and FYM respectively after 3 (d3) and 156  
 341 days (d156) of incubation: detection of SMX, N-ac-SMX and 9 other metabolites (M1-M9)  
 342 Results are expressed as % of extractable  $^{14}\text{C}$  or % of total  $^{14}\text{C}$ .  
 343 - Extracts non analyzed by HPLC because of a too low radioactivity

		SMX						N-ac-SMX					
		CONT		SGW		FYM		CONT		SGW		FYM	
		Extractable $^{14}\text{C}$ (%)	Total $^{14}\text{C}$ (%)	Extractable $^{14}\text{C}$ (%)	Total $^{14}\text{C}$ (%)	Extractable $^{14}\text{C}$ (%)	Total $^{14}\text{C}$ (%)	Extractable $^{14}\text{C}$ (%)	Total $^{14}\text{C}$ (%)	Extractable $^{14}\text{C}$ (%)	Total $^{14}\text{C}$ (%)	Extractable $^{14}\text{C}$ (%)	
HPCD	M6 (24.4')	0	0	8.7	1.8	10.3	1.8	27.8	6.4	0	0	0	
	SMX (25.4')	74.7	15.6	71.3	14.9	80.5	14.4	36.1	8.3	67.9	17.3	100	
	M7 (26.3')	18.6	3.9	13.2	2.8	0	0	24.2	5.5	32.1	8.2	0	
	N-ac-SMX (27.8')	0	0	6.9	1.4	0	0	11.9	2.7	0	0	0	
	M9 (37.3')	6.7	1.4	0	0	9.2	1.7	0	0	0	0	0	
	Total	100	20.9	100	20.9	100	17.9	100	22.9	100	25.5	100	
d3 MeCN pH9	M1 (3.7')	23.6	4.1	24.0	4.5	27.3	3.4	30.6	7.3	21.0	5.6	28.7	
	M2 (6.5')	37.8	6.6	27.1	5.0	20.9	2.6	20.1	4.8	20.0	5.4	17.6	
	M3 (9.8')	31.0	5.4	42.0	7.8	44.7	5.6	43.1	10.3	50.0	13.5	42.8	
	M9 (37.3')	7.7	1.3	7.0	1.3	7.2	0.9	6.2	1.5	8.8	2.4	11.0	
	Total	100	17.4	100	18.6	100	12.5	100	23.8	100	26.9	100	
MeCN pH2	M6 (24.4')	10.3	0.9	0	0	0	0	2.8	0.3	0	0	0	
	SMX (25.4')	46.1	3.9	100	7.7	76.7	5.2	74.7	6.9	87.4	8.2	69.9	
	N-ac-SMX (27.8')	0	0	0	0	0	0	12.5	1.2	12.6	1.2	10.1	
	M8 (35.0)	16.2	1.4	0	0	23.3	1.6	0	0	0	0	0	
	M9 (37.3')	27.4	2.3	0	0	0	0	10.1	0.9	0	0	20.0	
Total	100	8.4	100	7.7	100	6.8	100	9.2	100	9.4	100		
d156	MeCN	M1 (3.7')	66.7	10.1	67.1	11.9	69.6	11.6	71.2	13.1	61.0	9.4	58.8

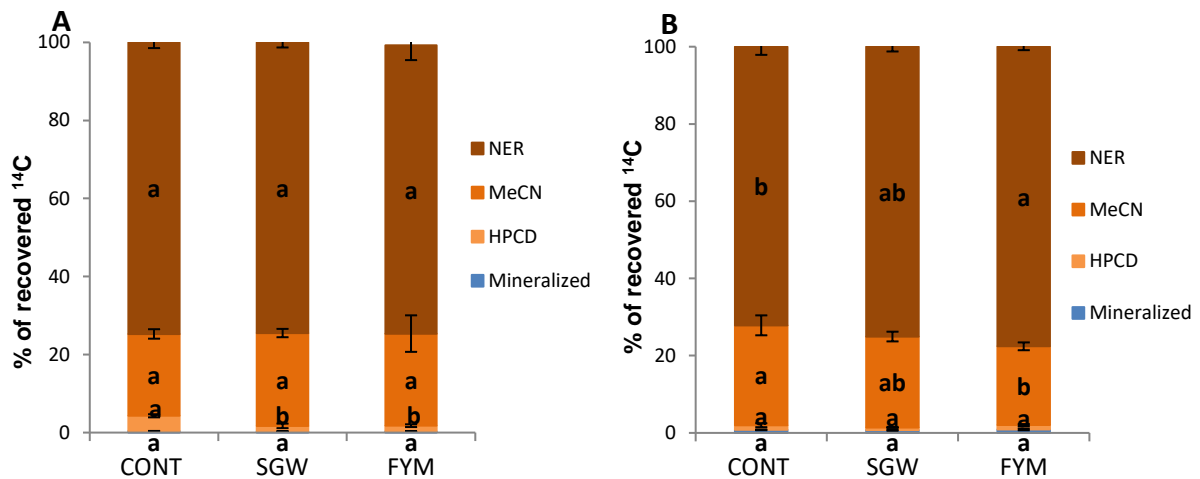
pH9	M2 (6.5')	12.4	1.9	0	0	0	0	7.8	1.4	0	0	12.9
	M5 (21.6')	0	0	16.2	2.9	17.0	2.8	0	0	19.5	3.0	22.3
	M9 (37.3')	20.9	3.2	16.7	3.0	13.5	2.3	21.0	3.9	19.6	3.0	6.0
	Total	100	15.2	100	17.8	100	16.7	100	18.4	100	15.4	100
MeCN pH2	M4 (16.7')	22.8	2.4	0	0							
	M6 (24.4')	66.0	7.0	43.8	3.5	-	-	-	-	-	-	-
	SMX (25.4')	11.2	1.2	56.2	4.4	-	-	-	-	-	-	-
	Total	100	10.6	100	7.9	-	-	-	-	-	-	-

344

345

346 3.2. Fate of CIP into soils amended or not with OWP

347 The <sup>14</sup>C balances ranged from 76 to 91.7% of added <sup>14</sup>C-CIP after 3 days and from 73.8 to  
 348 83.2% after 156 days. Presented results are expressed as percentages of recovered <sup>14</sup>C (Fig.  
 349 5).  
 350



351

352 **Fig. 5.** Distribution of <sup>14</sup>C-CIP in the different soil fractions (mineralized, HPCD and MeCN  
 353 extractable fractions, non-extractable residues), at (A) day 3, and (B) day 156, in the control  
 354 soil (CONT) and the soils amended with a sewage sludge-green waste compost (SGW) or  
 355 farmyard manure (FYM).  
 356

357

358 We observed few evolutions between d3 and d156 in all of the <sup>14</sup>C fractions. CIP  
 359 mineralization was considered as negligible since % of mineralization (0.5-0.6%) were below  
 360 the impurity threshold (1.6%) in the 3 soils after 156 days. The radioactivity present in the  
 361 HPCD fraction represented only a few percent of recovered <sup>14</sup>C. At d156, radioactivity was  
 362 concentrated in MeCN fractions (20.4-25.9%) and mainly in NER (72.2-77.6%). CIP became  
 363 rapidly strongly adsorbed or non-extractable. When comparing the 3 soils, NER were  
 364 significantly higher in FYM while MeCN residues were higher in CONT. SGW presented  
 intermediate values.

365

## 366 4. Discussion

### 367 4.1. Fate of SMX and N-ac-SMX

368 After addition of  $^{14}\text{C}$ -SMX and  $^{14}\text{C}$  N-ac-SMX as a solution, the fraction of easily extractable  
369  $^{14}\text{C}$  dropped in all soils till d3 and then decreased more slowly between 3 and 156 days. The  
370 decrease with time of SMX and N-ac-SMX in the HPCD fraction can be due to i) sorption on  
371 soil constituents, ii) physical sequestration in soil and/or iii) degradation. Then, radioactivity  
372 was quickly transferred to the hardly extractable fraction and NER. SMX and N-ac-SMX  
373 sorption affinity to soils was relatively low as shown by the  $K_d$  values in the 3 soils (1-3.2  
374  $\text{L}\cdot\text{kg}^{-1}$ ) that are in the same range of other studies (Drillia et al., 2005; Höltge and Kreuzig,  
375 2007; Kodešová et al., 2015). Different interactions may be involved in sorption of  
376 sulfonamides in soils, including surface complexation, ionic and hydrophobic interactions,  
377 some of them being pH-dependent (Leal et al., 2013; Tolls, 2001; Gao et al., 2010). In this  
378 study, soil pH ranged between 6.7 and 7.3, above the  $\text{pK}_a$ s of SMX and N-ac-SMX (Fig. S1),  
379 so that these molecules mainly existed in their anionic form, limiting ionic interactions with  
380 clay minerals (Gao et al, 2005). But sorption also depends on hydrophobicity, and SMX and  
381 its metabolites may thus interact with soil OM (Gao et al., 2010; Leal et al., 2013; Li et al.,  
382 2015). Thiele-Bruhn et al. (2004) highlighted the strong affinity between soil organic carbon  
383 and the sulfonamides. Furthermore, according to Martinez-Hernandez et al. (2016), sorption is  
384 fast and can play a key role during the first 48h of soil incubations. Gulkowska et al. (2014)  
385 attributed the fast formation of NER to chemical bonds between humic substances and the  
386 sulfonamide sulfamethazine. Thus sorption and chemical reactions of SMX and its  
387 metabolites could partially explain the high increase of  $^{14}\text{C}$  in the MeCN and non-extractable  
388 fractions at d3, leading to unavailability of these compounds to soil microorganisms. This  
389 hypothesis was also confirmed by HPLC analyses showing that after 3 days, SMX, N-ac-

390 SMX and other metabolites were partially transferred to the MeCN fraction. Then  
391 sulfonamides may desorb with time from this fraction to be transferred to readily extractable  
392 or non-extractable fractions (Drillia et al., 2005; Sukul et al., 2008). At the end of incubation,  
393 NER represented more than 50% of recovered  $^{14}\text{C}$  for SMX and N-ac-SMX. NER can have a  
394 chemical origin with binding of ATB and their metabolites to soil constituents (Gulkowska et  
395 al., 2014) but can also result from a physical sequestration of compounds or a microbial  
396 transformation of ATB and subsequent incorporation into microbial biomass (Kästner et al.,  
397 2014).

398 Previous work showed that SMX and N-ac-SMX are mainly degraded by soil microorganisms  
399 and that abiotic degradation is negligible (Acinnelli et al., 2007; Alvarino et al., 2016). Our  
400 results showed that SMX and N-ac-SMX biodegradation could be complete (~ 10% of  
401 mineralization at d156) or incomplete with metabolite production as previously showed by  
402 Liu et al. (2009) and Martinez-Hernandez et al. (2016). The production of metabolites, mainly  
403 more polar than parent compounds, started quickly after spiking soils with SMX and N-ac-  
404 SMX and confirmed results previously observed by Richardson and Ternes (2011) and Koba  
405 et al. (2017). Few metabolites were identified but the degradation pathway of SMX and N-ac-  
406 SMX path remained unclear. The main metabolite of sulfonamides is their acetylated form  
407 (Baran et al., 2011), whose formation can be reversible (Höltge & Kreuzig, 2007).  
408 Metabolites may also vary according to microbial species (Larcher and Yargeau, 2012) and  
409 environmental conditions (Müller et al., 2013).

410 Mineralization was significantly greater for N-ac-SMX than for SMX in the first days of  
411 incubation. These results are consistent with the lower adsorption and the higher extractability  
412 of N-ac-SMX in soil, leading to a higher availability of N-ac-SMX to soil microorganisms. At  
413 the opposite, the higher  $K_d$  of SMX can partially explain the higher production of abiotic  
414 NER. Höltge and Kreuzig (2007) previously observed higher  $K_d$  for SMX compared to N-ac-

415 SMX in most of soils and OWP they studied. Considering HPLC analyses, N-ac-SMX has  
416 almost completely disappeared from the HPCD extracts after 3 days and was present only in  
417 the MeCN pH2 fractions. The quantification of SMX as the most abundant molecule in the  
418 HPCD and MeCN pH2 extracts after N-ac-SMX addition in the 3 soils confirmed the  
419 reversible transformation of N-Ac-SMX to SMX (Höltge and Kreuzig, 2007; Zarfl et al.,  
420 2009).

421 The fate of SMX and N-ac-SMX also differs in the second part of incubation. For SMX, the  
422 production of MeCN and non-extractable residues was very fast up to 3 days and then evolved  
423 little between d3 and d156. For N-ac-SMX, hardly extracted residues increased till 3 days  
424 before decreasing. The production of NER was more progressive with N-ac-SMX than for  
425 SMX and was explained by a decrease of easily and hardly extractable fractions. Zarfl et al.  
426 (2009) suggested by simulation that NER were formed directly from easily extractable  
427 residues after sulfonamide addition. This would mean that some of the hardly extractable  
428 residues would go back to soil solution before contributing to the formation of NER.

429 Even if evolution with time was different for the 2 compounds, the HPCD, MeCN and non-  
430 extractable fractions were very similar at d156. For SMX and N-ac-SMX, radioactivity was  
431 mainly concentrated in NER and represented more than 50% of recovered  $^{14}\text{C}$ . Besides, Zarfl  
432 et al. (2009) also showed that the formation of NER from an acetylated metabolite of  
433 sulfadiazine was negligible. Therefore, deacetylation of N-ac-SMX to reform the parent  
434 compound should be necessary before its transfer to NER. Thus the transformation of N-SMX  
435 into SMX leads at long term to a similar distribution of radioactivity in extractable and non-  
436 extractable fractions for N-ac-SMX and SMX. On the other hand, the mineralization of the 2  
437 molecules started from the first days of incubation and explains why in this case, differences  
438 persisted over time.

439

#### 440 4.2. Fate of CIP

441 The fate of the fluoroquinolone CIP is very different from sulfonamides. No significant  
442 mineralization (less than 1%) was detected after 156 days. Except in artificial conditions, e.g.,  
443 pure cultures with optimal growth conditions, liquid media, bioreactor (Dorival-Garcia et al.,  
444 2013) or specific conditions with microorganisms from drinking water biofilter and adapted to  
445 high concentrations in CIP (Liao et al., 2016), most of the studies concluded that  
446 ciprofloxacin was recalcitrant to biodegradation (Girardi et al., 2011, Jechalke et al., 2014;  
447 Goulas et al., 2016).

448 The low CIP mineralization is due to its strong sorption and its resulting low bioavailability.  
449  $K_d$  of CIP was not measured in this study, but the literature reports very high values of  $K_d$ , >  
450  $500 \text{ kg.L}^{-1}$  (Leal et al., 2013; Vasudevan et al., 2009). Different mechanisms such as cation  
451 exchange, cation bridging, complexation, and H-binding may be responsible for sorption of  
452 fluoroquinolones in soils (Tolls, 2001; Vasudevan et al., 2009). At soil pH, zwitterionic  
453 species predominate in the soil solution. Hence a strong attraction between positive charges of  
454 CIP and negative charges of soil constituents can explain fast and strong CIP adsorption in  
455 soil. Drillia et al. (2005) measured very high desorption coefficients of fluoroquinolones,  
456 meaning that they mainly remained sorbed in the soil. Furthermore CIP addition in soil  
457 induces a high production of NER (Girardi et al., 2011). The decline of extractable CIP is thus  
458 mainly governed by sorption and formation of NER. In our study, from 3 days and throughout  
459 the incubation time, the radioactivity was concentrated in the hardly extractable fraction  
460 (20.4-25.9% at d156) and even more in NER (72.2-77.6% at d156). Girardi et al. (2011)  
461 comparing CIP degradation in biotic and abiotic conditions concluded that NER were mainly  
462 from abiotic origin.

463 But no mineralization does not mean no biodegradation. Due to the low radioactivity in the  
464 extracts, HPLC analyses could not be performed in this work. But other studies highlighted a



465 possible incomplete degradation and the appearance of metabolites (Girardi et al., 2011),  
466 especially in presence of fungi (Parshikov et al., 1999; Wetzstein et al., 1999).

467

#### 468 4.3. *Influence of soil properties on the fate of antibiotics*

469 The use of soils amended or not with OWP had for objective to study how long term  
470 applications of different OWP to soils impacted the fate of ATB. Two hypotheses were tested:

471 - OWP repeated application may increase ATB biodegradation (Accinelli et al., 2007)  
472 stimulating the soil indigenous microorganisms, introducing exogenous degrading  
473 microorganisms in soils or promoting the development of an adapted microflora due to the  
474 presence of antibiotics in OWP (Topp et al., 2013)

475 - OWP repeated application may enhance antibiotics sorption limiting their bioavailability and  
476 decreasing their mineralization (Li et al., 2015).

477 We found SMX and N-ac-SMX mineralization lower in OWP amended soils compared to  
478 CONT. Li et al (2015) previously observed that the addition of biosolids and compost to soil  
479 decreased SMX mineralization but the level of inhibition depended on the quantity of OM.

480 When comparing results of HPLC analyses between amended and unamended soils after  
481 SMX addition, the differences mainly appeared in the MeCN pH2 fraction, with less SMX  
482 and more metabolites present in CONT, as if the biodegradation was more advanced while  
483 remaining incomplete. These effects appeared at d3 and persisted over time. In soils incubated  
484 with N-ac-SMX, the main effect of amendments appeared in HPCD fractions, where organic  
485 matter seemed to have accelerated the de-acetylation into SMX and inhibited the formation of  
486 one metabolite (M6). The M5 metabolite present at d156 after addition of SMX and N-ac-  
487 SMX seemed specifically produced in amended soils.

488 On another side, differences were observed between SGW and FYM, i.e., SMX and N-ac-  
489 SMX mineralization was more inhibited in SGW than in FYM. HPLC analyses also showed

490 that the proportion of easily extractable SMX was higher in FYM while strongly adsorbed  
491 SMX was higher in SGW. These results are consistent with soil organic carbon contents  
492 higher in SGW than in FYM (17.6 vs 15.5 g.kg<sup>-1</sup>) and values of K<sub>d</sub> higher in SGW than in  
493 FYM for both SMX and N-ac-SMX. Furthermore, Racke and Lichtenstein (1985) reported  
494 that the quality of OM and its degree of decomposition were also key factors. OM is more  
495 stable and less degradable in compost of sewage sludge and green waste than in manure.  
496 Finally organic amendments stimulated the production of NER whatever the studied antibiotic  
497 (SMX, N-ac-SMX, CIP). The increase of NER occurred very quickly with SMX and CIP and  
498 later with N-ac-SMX. In addition, this effect was more pronounced in FYM than in SGW. Li  
499 et al. (2015) also showed that OWP amendment increased the formation and accumulation of  
500 NER in a soil spiked with SMX from the first days of incubation. They suggested that OWP  
501 provide additional sites facilitating binding or conjugation interactions between contaminants  
502 and soil constituents and/or promoted microbial transformation leading to the formation of  
503 intermediates that were readily bound to the matrix.

504

## 505 **5. Conclusion**

506 The fate of two sulfonamides, SMX and N-ac-SMX, and one fluoroquinolone, CIP, in soils  
507 amended or not with manure or a co-compost of sludge and green waste depends on  
508 molecules and soil characteristics. Antibiotic mineralization was nil or low after 156 days  
509 (CIP << SMX < N-ac-SMX) while the production of NER accounted for more than 50% of  
510 recovered <sup>14</sup>C.

511 Organic amendments decrease antibiotics degradation and mineralization (SMX and N-ac-  
512 SMX) and increase the production of NER (SMX, N-ac-SMX and CIP). The effects also  
513 depend on the quantity and quality of added OM. The SGW compost further inhibits  
514 mineralization while FYM enhances the production of NER. Land application of OWP is an

515 increasing practice in agriculture and urban gardening and so the impact of OWP on the fate  
516 of ATB and its associated health and environmental risks are of great interest to be studied.  
517 But OWP often contain multiple contaminants (other xenobiotics, metals ...), as well as a  
518 complex organic matrix that interacts with the contaminants during storage or treatment  
519 (composting, methanisation, liming, drying...), and then after soil spreading. Consequently it  
520 would be interesting to evaluate whether the concomitant addition of contaminants (cocktail  
521 effects) in soils and/or the evolution of OM-ATB interactions over time have an impact on the  
522 fate of antibiotics.

523

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531

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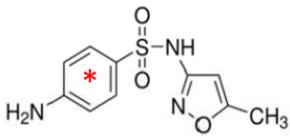
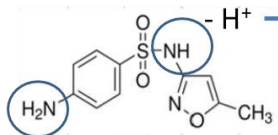
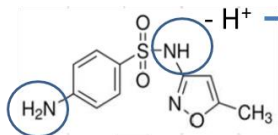
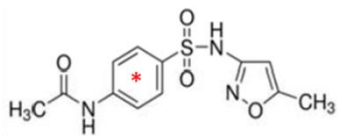
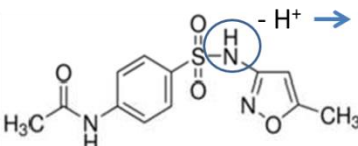
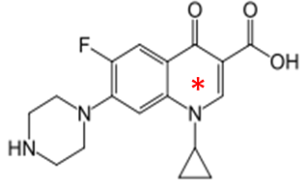

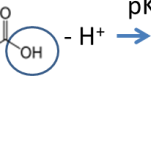
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718 **Appendix A. Supplementary data**

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720 **Fig. S1.** Molecular structure, pKa and labelling of sulfamethoxazole, N-acetyl-

721 sulfamethoxazole and ciprofloxacin (\*  $^{14}\text{C}$  labelling)

Compounds	Molecular structure	Molecular speciation
Sulfamethoxazole (SMX)		<p>pKa 1.7</p> <p>+ H<sup>+</sup> → </p> <p>pKa 5.6</p> <p>- H<sup>+</sup> → </p>
N-acetyl-Sulfamethoxazole (N-ac-SMX)		<p>pKa 5.6</p> <p>- H<sup>+</sup> → </p>
Ciprofloxacin (CIP)		<p>pKa 8.8</p> <p>← - H<sup>+</sup> </p> <p>pKa 6.2</p> <p>- H<sup>+</sup> → </p>

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726 **Table S1**

727 Selected extracts for qualitative analyses of radioactivity by HPLC

Molecule	Soil	Day	HPCD extractions		MeCN pH9 extractions		MeCN pH2 upper phase extractions	
			Concentration	Pool	Concentration	Pool	Concentration	Pool
N-ac-SMX	CONT SGW FYM	3	SPE cartridge strata XA type	no	Evaporation	no	Evaporation	yes
N-ac-SMX	CONT SGW FYM	156			Evaporation	no	Evaporation	yes
SMX	CONT SGW FYM	3	SPE cartridge strata XA type	no	Evaporation	no	Evaporation	yes
SMX	CONT SGW FYM	156			Evaporation	no	Evaporation	yes

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729

730 **Table S2.** Ratio between percentages of  $^{14}\text{C}$ -SMX and  $^{14}\text{C}$ - N-ac-SMX in the mineralized,  
 731 HPCD, MeCN and non-extractable fractions at day 3 and day 156.

732 \* means that the percentages of radioactivity in the considered fraction are significantly  
 733 different for SMX and N-ac-SMX.

	SMX/N-ac-SMX							
	Mineralized fraction		HPCD extractable fraction		MeCN extractable fraction		Non extractable fraction	
	d3	d156	d3	d156	d3	d156	d3	d156
CONT	0.6 *	0.8 *	0.9 *	0.9	0.7 *	0.9	1.4 *	1.1 *
SGW	0.5 *	0.7 *	0.8 *	1.1 *	0.7 *	1.0	1.4 *	1.0
FYM	0.6 *	0.9	0.7 *	1.1 *	0.6 *	1.1	1.7 *	1.0

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