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► **To cite this version:**

Marine Roulier, Loic Carasco, Isabelle Le Hécho, Maïté Bueno, Daniel Orjollet, et al.. Iodine distribution and volatilization in contrasting forms of forest humus during a laboratory incubation experiment. *Journal of Environmental Radioactivity*, 2022, 248, pp.106872. 10.1016/j.jenvrad.2022.106872 . irsn-04065105

**HAL Id: irsn-04065105**

<https://irsn.hal.science/irsn-04065105v1>

Submitted on 11 Apr 2023

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# Iodine distribution and volatilization in contrasting forms of forest humus during a laboratory incubation experiment

## Abstract

Radionuclides  $^{129}\text{I}$  ( $t_{1/2} = 15.7 \times 10^6$  years) and  $^{131}\text{I}$  ( $t_{1/2} = 8.02$  days) are both introduced into the environment as a result of nuclear human activities. Environmental transfer pathways and fluxes between and within ecosystems are essential information for risk assessment. In forest ecosystems, humus degradation over time could result in re-mobilization and then downward migration and/or volatilization of intercepted  $^{129}\text{I}$ . In order to estimate the scale of these processes, humus (mull and moder forms) sampled under deciduous and coniferous forests were spiked with  $^{125}\text{I}^-$  ( $t_{1/2} = 59.4$  days), as a surrogate for  $^{129}\text{I}$ , in order to study the evolution of its water-soluble and organic fractions as well as the volatilization rate during humus degradation at laboratory scale. To our knowledge, this is the first time that interactions between iodine and contrasting forms of forest humus have been investigated. The evolution of native stable iodine ( $^{127}\text{I}$ ) pools in unspiked humus was also studied.

The nature of the humus' organic matter appears to be a factor that impacts on the proportions of water-soluble and organic fractions of iodine and on their evolution. Iodine-125 was mainly organically bound (fraction for mulls and moders: ~54-59 and 41-49%, respectively) and no clear evolution was observed within the 4-month incubation period. A large decrease in  $^{125}\text{I}$  water-solubility occurred, being more marked for mull (from ~14-32 to 3-7%) than for moder (from ~21-37 to 7-19%) humus. By contrast, a significant fraction was not extractible (~38-43%) and varied in inverse proportion to the water-soluble fraction, suggesting a stabilisation of iodine in humus after wet deposit. The nature of the humus organic matter also impacted on  $^{125}\text{I}$  volatilization. Although of the same order of magnitude, the total volatilization of  $^{125}\text{I}$  was higher for moders (~0.039-0.323%) than for mulls (~0.015-0.023%) within the 4-month incubation period. Volatilization rates for mulls were correlated with the water-soluble fraction, implying that volatilization of  $^{125}\text{I}$  could occur from the humus solution. Our results

27 showed that humus is thus a zone of iodine accumulation by association with organic matter  
28 and that potential losses by lixiviation are significantly more important compared to  
29 volatilization.

30

31 **Keywords:** Iodine; solubility; volatilization; organic matter; forest

32

### 33 **1. Introduction**

34 Natural iodine (I) is composed of  $^{127}\text{I}$ , its single stable isotope, and the long-lived  
35 radionuclide  $^{129}\text{I}$  with a half-life of  $15.7 \times 10^6$  years. Iodine-129 and iodine-131 ( $t_{1/2} = 8.02$  days)  
36 are both introduced into the environment as a result of nuclear human activities (e.g.  
37 atmospheric releases following nuclear accidents, reprocessing of radioactive wastes, and in the  
38 medical field).  $^{131}\text{I}$  represents a significant and immediate health hazard associated with large-  
39 scale nuclear events (UNSCEAR, 2011); while  $^{129}\text{I}$ , due to its extremely long half-life, is one  
40 of the most persistent anthropogenic radionuclides in ecosystems representing a challenge in  
41 terms of long-term management (Hou et al., 2009).

42 Among ecosystems liable to be affected by radioiodine atmospheric inputs, forests are  
43 of major interest because of their large and complex contact surfaces with the atmosphere.  
44 These ecosystems can be both a sink for elements via their interception and accumulation, and  
45 also a secondary source via their re-emission to the atmosphere and/or drainage to neighboring  
46 ecosystems (Ranger et al., 1995; Shaw, 2007). Our previous studies of the distribution of iodine  
47 in French forest soils have shown that humus could act as a temporary store of iodine from  
48 atmospheric deposition and litterfall, since the net iodine accumulation rates in humus could  
49 account for up to 82.3% of annual iodine input to the soil (Roulier et al., 2018, 2019). Because  
50 the humus layer is in a key position as a transition zone between the atmosphere and the soil, a  
51 better understanding of iodine behavior in this forest compartment would help improve

52 estimated fluxes through its interfaces, essential for relevant modelling of iodine dynamics in  
53 forests. Humus is made up of the organic matter part of the soil resulting from chemical and  
54 microbiological decomposition of vegetal deposits. This organic matter layer is composed of  
55 humic substances (i.e., fulvic and humic acids, humins) and macro-, meso- and micro-fauna.  
56 Mull humus form is subject to rapid degradation of litter linked to high biological activity, while  
57 moder and mor forms are characterized by a lower to almost nil biological activity, resulting in  
58 intermediate to extremely low turnover of organic matter, respectively (Baize et al., 2009).

59 Iodine occurs in several oxidation states in the environment as inorganic (iodide ( $I^-$ ) and  
60 iodate ( $IO_3^-$ )) and organic species. Metal oxides and hydroxides (e.g.  $Fe(OH)_3$ ,  $Al(OH)_3$  and  
61  $MnO_2$ ) can play a role in iodine retention in soils due to their amphoteric sites (Dai et al.,  
62 2004; Muramatsu et al., 1990; Shetaya et al., 2012; Whitehead, 1973, 1978). Although the link  
63 between iodine and soil organic matter (OM) has been reported in various studies (e.g. Bostock  
64 et al., 2003; Dai et al., 2009; Roulier et al., 2019; Santschi et al., 2017; Schwehr et al., 2009;  
65 Shimamoto et al., 2011; Steinberg et al., 2008a, 2008b; Whitehead, 1973; Xu et al., 2012), few  
66 studies have focused specifically on the humus compartment (Bostock et al., 2003; Söderlund  
67 et al., 2017; Takeda et al., 2015). Mechanisms governing the incorporation of inorganic iodine  
68 into organic matter have been the subject of several studies. Iodate and iodide have been shown  
69 to be reduced and oxidized, respectively, to reactive intermediate species ( $HIO$  and  $I_2$ ) reacting  
70 with organic molecules possessing electron donating groups such as phenols (Franke et Kupsch,  
71 2010; Fukui et al., 1996; Reiller et al., 2006; Schlegel et al., 2006; Warner et al., 2000;  
72 Whitehead, 1974). Additionally, Schlegel et al. (2006) showed that iodine was linked to  
73 aromatic rings in naturally iodinated humic substances. The iodination of amines through the  
74 formation of iodine–nitrogen bonds from amino groups in organic matter has also been  
75 demonstrated both in synthetic solutions (Franke and Kupsch, 2010) and in humic substances  
76 extracted from soil (Xu et al., 2013). Iodine in humus can be leached during rainy events, which

77 may affect its distribution in soils, groundwater, surface water and vegetation. Studies based on  
78 chemical extractions to evaluate the importance of the water-soluble fraction of iodine in soils  
79 have shown that it may be significant (up to 25% of total soil iodine) (e.g. Ashworth et al.,  
80 2003; Bostock et al., 2003; Hansen et al., 2011; Hou et al., 2003; Johnson, 1980; Qiao et al.,  
81 2012; Takeda et al., 2015). In addition, although organic matter could decrease the water-  
82 solubility of soil iodine, its dissolved fraction may also be a source of dispersion of iodine due  
83 to the formation of colloidal or dissolved organic material (Chang et al., 2014; Unno et al.,  
84 2017; Xu et al., 2011a, b). The importance of the link between iodine and dissolved organic  
85 matter has been reported by Xu et al. (2011a) explaining that a small and easily mobilized  
86 fraction of OM could behave as a source of iodine under colloidal organic form.

87         Regarding iodine volatilization, data in the literature are available only for the soil  
88 compartment. A fraction of iodine in soil can be volatilized as I<sub>2</sub> or CH<sub>3</sub>I, following abiotic  
89 and/or biotic processes (e.g. Allard et al., 2010; Keppler et al., 2003; Muramatsu and Yoshida,  
90 1995). Sheppard et al. (1994) determined <sup>125</sup>I volatilization from peat soil in laboratory  
91 experiments and found a loss of only 0.07% over 66 days after addition for a spiking of about  
92 7 kBq g<sup>-1</sup>. Similarly, Bostock et al. (2003) found a minor <sup>125</sup>I loss by volatilization (total loss  
93 of 0.011% over 22 days for a spike of approximately 1 kBq g<sup>-1</sup>) from forest soils, due to the  
94 high retention of iodine by OM. Although the amounts of volatile iodine reported from  
95 laboratory experiments appear low, Sive et al. (2007) identified forests as a terrestrial source of  
96 methyl iodide (CH<sub>3</sub>I) in the North Carolina atmosphere.

97         Considering the lack of information regarding iodine re-mobilization during humus  
98 degradation and subsequent downward migration and/or volatilization, we conducted  
99 laboratory incubation experiments with an <sup>125</sup>I tracer in an attempt to quantify these processes.  
100 In this study, <sup>125</sup>I was chosen in place of <sup>129</sup>I to facilitate direct measurements using gamma  
101 spectrometry, enabling us to work at very low concentration levels and quantify volatilization

102 rates. Although iodine exists in the environment in the form of iodide and iodate, additions of  
 103  $^{125}\text{I}$  were conducted only using iodide form. Under well-controlled conditions and considering  
 104 six humus samples from coniferous and deciduous forests including mull and moder forms, our  
 105 aims were to: 1) examine the evolution over time of the distribution of the iodine tracer between  
 106 water-soluble and organic fractions, 2) compare the tracer dynamics to those of naturally-  
 107 occurring stable iodine, and 3) estimate the volatilization rate of the iodine tracer occurring in  
 108 parallel with its re-distribution.

109

## 110 **2. Materials and methods**

### 111 **2.1. Humus sampling**

112 Six humus samples were taken from four deciduous (Oak: CHP40 and CHS35; Beech:  
 113 HET25 and HET29) and two coniferous (Silver fir: SP25 and SP63) forests of the French  
 114 RENECOFOR network (the National Network for the long-term Monitoring of Forest  
 115 Ecosystem) managed by the ONF (the French National Forest Board)  
 116 (<http://www.onf.fr/renecofor>). Different forest species were chosen because of the wide variety  
 117 of plant debris that can be supplied to the soil impacting the nature of the humus OM. The  
 118 localtion of these six sampling sites is shown in Supplementary Information (SI) Figure S1.  
 119 Humus samples were representative of two extreme levels of OM turnover (classification  
 120 according to Brêthes and Ulrich (1997)), i.e. fast (eumull and mesomull) and slow (dysmoder  
 121 and moder) turnover (Table 1).

122

123 Table 1. Sites descriptions for the six humus samples studied.  $t_{\text{resDM}}$  corresponds to dry mass  
 124 residence time in humus.

<b>Plot</b>	<b>Type of tree species</b>	<b>Dominant tree species</b>	<b>Type of humus</b>	<b><math>t_{\text{resDM}}</math> (year)<sup>1</sup></b>	<b>Level of OM turnover</b>
CHP40	Deciduous	Oak	Eumull	1.1	Fast
CHS35	Deciduous	Oak	Dysmoder	16.8	Slow
HET25	Deciduous	Beech	Eumull	3.1	Fast

HET29	Deciduous	Beech	Dysmoder	16.8	Slow
SP25	Coniferous	Silver Fir	Mesomull	4.8	Fast
SP63	Coniferous	Silver Fir	Moder	12.5	Slow

<sup>1</sup>from Roulier et al. (2019)

125

126           Sampling was carried out from 2 to 20 October 2017. About 2 kg (wet mass) of humus,  
127 carefully separated by hand, were sampled (height of sampling corresponding to a few  
128 centimetres above the mineral soil) and kept in closed plastic bags. An additional sorting was  
129 performed in the laboratory to remove moss and branch debris, then humus were cut with a  
130 chisel (size of pieces: ~1cm) before measuring their field water content (calculated as the ratio  
131 of the weight of water to the weight of dry humus). Water content varied between 100 and  
132 300%, close to that used by Cotrufo et al. (2010) to quantify litter decomposition rates. Samples  
133 were stored at 4°C before being spiked with iodide-125.

134

## 135 **2.2 Set-up and monitoring of the incubation experiment**

### 136 **2.2.1. Preparation of humus samples: <sup>125</sup>I spiked and unspiked samples**

137           The six humus samples were spiked with the radioisotope <sup>125</sup>I ( $t_{1/2} = 59.9$  days) in iodide  
138 (I<sup>-</sup>) form. The spike solution was prepared by a 1000-fold dilution of certified solution (LEA,  
139 ORANO) with ultra-high quality (UHQ) water (Millipore, >18.2 MΩ·cm), giving a chemical  
140 composition of 15 pg g<sup>-1</sup> (10 kBq) of <sup>125</sup>I, 50 ng g<sup>-1</sup> of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> and 50 ng g<sup>-1</sup> of NaI. Between  
141 152 and 181 g (dry weight, dw) of humus were placed in a plastic tray and contaminated  
142 uniformly with the spiking solution (approx. 40 kBq g<sup>-1</sup> dw of <sup>125</sup>I (62 pg g<sup>-1</sup>)). To obtain a  
143 homogeneous contamination of the humus, these were saturated in solution (uniform addition  
144 of spiking solution volume until water saturation). The spiked humus samples were air-dried  
145 until the target water content of the humus was reached, with final water content of approx.  
146 300-800% compared to dry humus weight. This was then mixed using a metal spatula. The  
147 homogeneity of the spiked humus was estimated by comparing expected theoretical activity

148 with actual activity measured. Two aliquots of each spiked humus samples were analysed using  
149 gamma spectrometry after drying at 40°C in an oven. This low drying temperature was used to  
150 prevent the volatilization of iodine at higher temperatures. The mean difference between  
151 expected activity and measured activity was  $11 \pm 9 \%$ , which was close to the variability  
152 obtained between duplicates ( $10 \pm 4 \%$ ) and the measurement uncertainty ( $\approx 10 \%$ ). After  
153 sampling for time zero (t-0) measurements (see §2.4.) and validating sample homogeneity, the  
154 six spiked humus samples were transferred into 2 L glass bottles, in duplicate (named samples  
155 R1 and R2 for each sample) using a metal spatula. The twelve incubation bottles were then  
156 placed in the incubation chamber and connected to the activated carbon traps (Figure 1; see  
157 §2.2.2.). The incubation bottles were periodically shaken by hand ( $\sim$  once a week) to  
158 homogenize water content. Their weights were monitored and, when needed, additional UHQ  
159 water was added to keep the targeted initial water content constant (SI Figure S2).

160 Unspiked humus samples used to monitor the evolution of naturally present stable  
161 iodine were treated following the same procedure, using UHQ water to reach the targeted initial  
162 water content. Due to expected low volatilized iodine concentrations and the difficulty in  
163 extracting stable iodine from the activated carbon traps, stable iodine volatilization was not  
164 monitored. The humus samples were sampled for t-0 measurements and transferred into six  
165 incubation bottles of 1 L using a metal spatula. For each sampling time, duplicate samples were  
166 taken from each bottle (named samples R3 and R4). The incubation bottles were also  
167 periodically shaken and their weights were monitored and, when needed, additional UHQ water  
168 was added to keep the targeted initial water content constant (SI Figure S2).

169

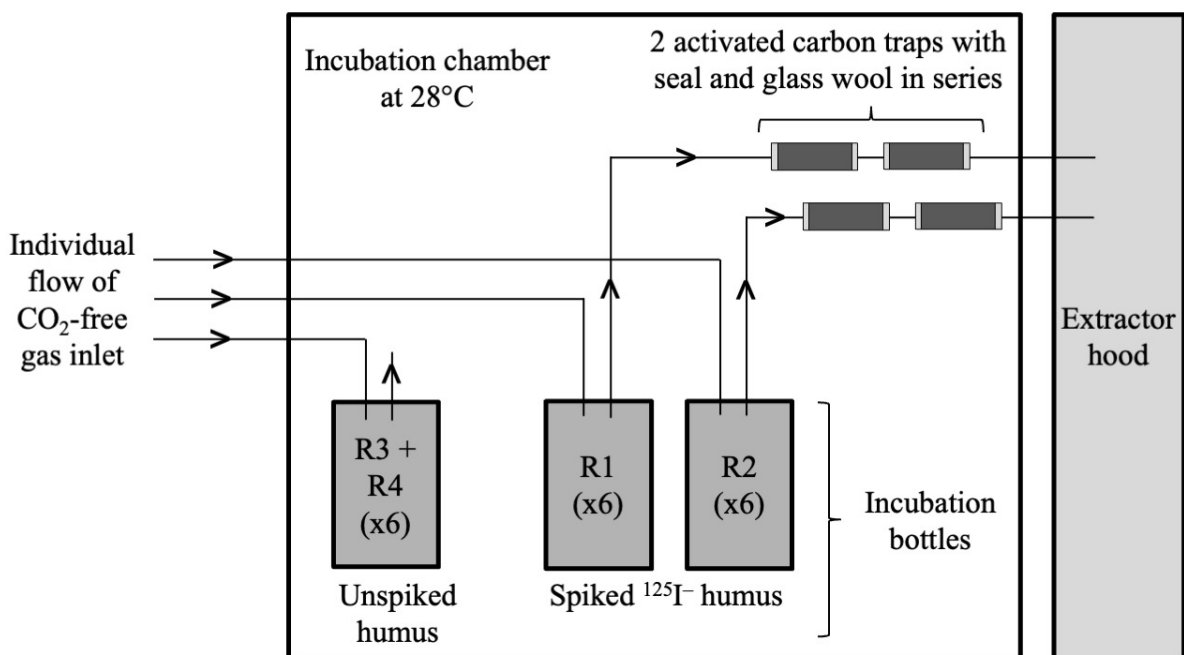
### 170 **2.2.2. Incubation design**

171 Figure 1 illustrates the experimental incubation system. The 18 bottles containing spiked  
172 and unspiked humus were placed in incubation chambers kept at a temperature of 28°C to



173 optimise the humus decomposition rate (Pietikäinen et al., 2005). Each incubation bottle was  
174 connected to a CO<sub>2</sub>-free air gas inlet that was calibrated at 20 ± 2 mL min<sup>-1</sup>. Volatile <sup>125</sup>I  
175 produced within the incubation period was trapped at the system outlet on activated carbon  
176 (CHEMVIRON CARBON, nuclearcarb, grade 207B 1.5KI) traps conditioned in glass tubes  
177 (~7g per trap) with seal and glass wool. Two activated carbon traps were placed in series to  
178 ensure total trapping of volatile iodine.

179



180

181

182

Figure 1. The experimental incubation system.

### 183 2.2.3. Humus and activated carbon traps sampling

184 The incubation bottles of spiked humus were periodically opened to sample about 5 g  
185 (dw) of humus for measurement (n = 1 per incubation bottle). About 2.5 g were dedicated to  
186 water content determination, and subsequent measurements of total <sup>125</sup>I activity and carbon-to-  
187 nitrogen (C/N) ratio after drying at 40°C were performed (see §2.4.). About 2 g (dw) were  
188 subjected to successive extractions with UHQ water and tetra methyl ammonium hydroxide  
189 (TMAH) (see §2.3.1.). About 0.5 g (dw) was used to determine total <sup>125</sup>I using the same protocol

190 used to determine  $^{127}\text{I}$  in order to estimate extraction efficiency (see §2.3.2.). These samplings  
191 were performed six times after  $t=0$ , i.e. at 7, 14, 35, 56, 84 and 112 days of the incubation  
192 (referred to as  $t-0$ ,  $t-7$ ,  $t-14$ ,  $t-35$ ,  $t-56$ ,  $t-84$  and  $t-112$ , respectively).

193 Unspiked humus were sampled only at the end of incubation period to perform the same  
194 analyses (except for radiotracer activity). Sampling was performed in duplicate for each  
195 incubation bottle (R3 and R4).

196 During the incubation period, the active carbon traps were sampled at intervals of days  
197 at the beginning of incubation to weeks at the end of incubation. The activated carbon traps  
198 were transferred from the glass tube to a plastic tube for  $\gamma$ -spectrometry measurements (see  
199 §2.4.1). The  $^{125}\text{I}$  volatilization rate ( $\text{d}^{-1}$ ) was defined as the ratio of  $^{125}\text{I}$  activity measured in the  
200 activated carbon traps during the sampling time ( $\text{Bq d}^{-1}$ ) and total activity of  $^{125}\text{I}$  in the  
201 incubation bottle (Bq).

202

## 203 **2.3. Samples treatment for total and chemical extractions of iodine**

### 204 **2.3.1. Successive chemical extractions of humus iodine**

205 A sequential extraction procedure was used to estimate pools of water-soluble iodine  
206 (i.e. w-soluble fraction) and iodine associated with OM (i.e. organic fraction) in humus (Hansen  
207 et al., 2011; Shetaya et al., 2012). At each sampling time, the target iodine pools were obtained  
208 after 1) three successive extractions with UHQ water (w-soluble fraction), followed by 2) three  
209 successive extractions with 5% TMAH (organic fraction) at room temperature, as previously  
210 optimised for soil samples (Roulier et al., 2018). Final  $^{125}\text{I}$  and  $^{127}\text{I}$  concentrations in the solid  
211 and solution obtained at each stage of the three successive extractions were plotted and fitted  
212 with a linear regression model to generate y-intercepts corresponding to the percentage of the  
213 considered available  $^{125}\text{I}$  and  $^{127}\text{I}$  fraction (Teramage et al., 2018). About 9-15 g of wet humus  
214 were weighed in a 50 mL polypropylene sterile centrifuge tube, then a given volume of

215 extracting solution (UHQ water or 5% TMAH, Sigma-Aldrich, Germany, 25 wt% in H<sub>2</sub>O) was  
216 added (i) to keep wet humus in constant contact with the solution, and (ii) to maintain the same  
217 v/w ratio (dry weight) in successive extractions (volume ranged between 18 and 30 mL  
218 according to humus sample). For each extraction step, tubes were mechanically stirred at room  
219 temperature for 3 h at 300 rpm (IKA, KS250). After centrifugation at 10,000 g for 25 min (w-  
220 soluble fraction) or 40 min (organic fraction), supernatants were filtered (0.22 µm, PES filter)  
221 and stored at 4°C until analysis was performed. Reagent blanks were subjected to the same  
222 procedure. The residual fraction was calculated as the difference of total iodine in the humus  
223 minus the sum of w-soluble and organic fractions.

224

### 225 **2.3.2. Total iodine extraction**

226 Alkaline extraction using TMAH was performed to determine total <sup>127</sup>I in unspiked  
227 humus samples (Shetaya et al., 2012; Watts and Mitchell, 2008). Approximately 0.5 g dry mass  
228 of humus was weighed directly into a 50 mL DigiTUBE (polypropylene) and mixed with 10  
229 mL of 5% TMAH. Reagent blanks were added in each digestion run. Tubes were placed at  
230 90°C for 3h on a sand bath (t-0) or in a temperature-controlled digestion system (DigiPREP,  
231 SCP Science) (t-112). After heating, solutions were diluted to 20 mL using UHQ water. After  
232 settling overnight, digested samples were filtered at 0.22 µm (PES filter) and stored at 4°C until  
233 analysis by inductively coupled plasma mass spectrometry (ICP-MS) for <sup>127</sup>I (see §2.4.1). The  
234 same procedure was used for <sup>125</sup>I spiked humus in order to estimate extraction efficiency by  
235 comparing it with direct solid gamma spectrometry analysis. Extraction efficiency ranged from  
236 70 to 85% and was used to calculate total <sup>127</sup>I concentrations in humus at t-0 and t-112.

237

## 238 **2.4. Iodine, pH, carbon, nitrogen and microbial activity measurements**

### 239 **2.4.1. Radioiodine (<sup>125</sup>I) and stable iodine (<sup>127</sup>I)**

240 The  $^{125}\text{I}$  analysis for both liquid extracts and solid samples was carried out using a hyper  
241 pure germanium gamma spectrometer (Itech Instruments, GCD 30-190X). All activity  
242 measurements were decay-corrected back to 1 December 2017. The detection limit was around  
243 0.3 Bq per sample ( $0.015 \text{ Bq mL}^{-1}$  for a 20 mL sample) and analytical uncertainty was around  
244 4% ( $1\sigma$ ).

245 The stable iodine analysis was performed using an Agilent 7500ce ICP-MS (Agilent  
246 Technologies, Tokyo, Japan) using external calibration in UHQ water (water samples) or 0.05%  
247 TMAH (organic fraction and total iodine extraction). The instrument detection limits of iodine  
248 ranged between 0.07 and  $0.89 \mu\text{g L}^{-1}$  depending on the sample matrix. Analytical uncertainty  
249 was  $<5\%$  (relative standard deviation (RSD), 10 replicates).

250

#### 251 **2.4.2. pH, total carbon and nitrogen, and dissolved organic carbon**

252 pH was measured in the first water extract ( $\text{pH}_{\text{water}}$ ). The carbon and nitrogen contents  
253 of the solid samples were determined using a CHNS Elemental Analyzer (Elementar, Vario El  
254 cube). The quantity and quality of organic matter were determined using a microplate reader  
255 (TECAN M1000). UV-absorbance measurements (of the three extracts with UHQ water and  
256 TMAH) were used for organic carbon determination (Carter et al., 2012) and fluorescence  
257 measurements (for the first extract with UHQ water and TMAH) ( $\lambda_{\text{ex}}370 \text{ nm}$  and  $\lambda_{\text{em}}450$  and  
258  $500 \text{ nm}$ ) to calculate fluorescence index (FI) as the ratio of emission intensity at 450 nm to that  
259 at 500 nm for a fixed excitation wavelength of 370 nm (Johnson et al., 2011; McKnight et al.,  
260 2001). The FI is commonly employed as an indicator of dissolved organic carbon quality.  
261 McKnight et al. (2001) demonstrated that the FI decreased as a function of aromaticity for fulvic  
262 acid samples.

263

#### 264 **2.4.3. Microbial activity**

265 As OM degradation (e.g., Pietikäinen et al., 2005) and iodine volatilization (i.e.,  
266 Amachi, 2008; Muramatsu et al., 2004) can be due to microbial activity, community-level  
267 physiological profiles (CLPP) were assessed using the Biolog EcoPlate™ (Biolog, Hayward,  
268 CA, USA). These contain 31 of the most useful carbon sources (C-sources) for soil community  
269 analysis in triplicate and three controls with no substrate. The formation of purple colouring  
270 and an increase in optical density at 590 nm (OD<sub>590</sub>) occur when microorganisms use the carbon  
271 source and begin to respire (Leflaive et al., 2005; Biolog data sheet). About 1 mL of the first  
272 water-extract (n = 1) was recovered prior to filtration and diluted 5-fold to give a w/v ratio of  
273 1/100 (Pohlad and Owen, 2009) with 0.22 µm filtered UHQ water (PES). For each plate, 150  
274 µL of water-extracts of two humus samples and one blank were inoculated into microplate wells  
275 and incubated at 25°C in the dark for up to 96 hours. The OD<sub>590</sub> was measured every 24 hours  
276 for 4 days using a microplate reader set to a wavelength of 590 nm (TECAN M1000). The  
277 OD<sub>590</sub> measured was corrected by subtracting the value of the respective control well (net  
278 OD<sub>590</sub>). Negative values that occasionally resulted were set to zero. The final reading time was  
279 chosen by counting the number of wells at time point ( $n_t$ ) for which net OD<sub>590</sub> exceeds 0.1. The  
280 time with the greatest change (i.e.  $n_t - n_{t-1}$  was maximum) was selected (Glimm et al., 1997;  
281 Grove et al., 2004). After counting for overall plates and communities, the reading time of 48  
282 h was thus selected. In order to evaluate changes in CLPP over incubation time and among  
283 different forms of humus, the number of positive C-sources, i.e. with a net OD<sub>590</sub> above of 0.4,  
284 was enumerated (Colinon-Dupuich et al., 2011; Verschuere et al., 1997). The substrates were  
285 also assigned to guilds according to their chemical nature (Choi and Dobbs, 1999; SI Table S1).  
286 Following the procedure described by Leflaive et al. (2005), guilds were equally weighted to  
287 the largest one (carbohydrates, 10 substrates) from Eq. 1 for the guild x with  $n$  substrates:

288 
$$S_x = \frac{10}{n} \times \sum_{i=1}^n \text{net OD}_{590} (\text{substrate } i) \quad (\text{Eq. 1})$$

289 To factor in differences in guild size, the calculated value  $S_x$  was divided by their sum resulting  
290 in the new value  $P_x$  (Eq. 2):

$$291 \quad P_x = \frac{S_x}{\sum_{i=1}^6 S_i} \quad (\text{Eq. 2})$$

292

## 293 **2.5. Modelling the kinetic evolution of iodine pools**

294 The evolutions of  $^{125}\text{I}$  w-soluble, organic and residual fractions over time were modelled  
295 according to a first kinetic law in the following form (Eq. 3):

$$296 \quad y = (A_0 - A_f) \times e^{-kt} + A_f \quad (\text{Eq. 3})$$

297 Where  $A_0$  = percentage of the  $^{125}\text{I}$ -pool considered at t-0;  $A_f$  = final percentage of the  $^{125}\text{I}$ -pool  
298 considered once stabilization is reached;  $k$  = rate coefficient ( $\text{day}^{-1}$ );  $t$  = incubation time (day).

299 In line with Bostock et al. (2003) and their modelling of iodine volatilization rates over  
300 time using a first kinetic law, the cumulative fluxes of volatilized  $^{125}\text{I}$  were modelled with using  
301 first kinetic law in the following form (Eq. 4):

$$302 \quad A_t^v = A_f^v \times (1 - e^{-k_v t}) \quad (\text{Eq. 4})$$

303 Where  $A_t^v$  = cumulative volatilized  $^{125}\text{I}$  at time t (mol);  $A_f^v$  = maximum cumulative of volatilized  
304  $^{125}\text{I}$  (mol);  $k_v$  = rate coefficient ( $\text{day}^{-1}$ );  $t$  = incubation time (day).

305 Equations 3 and 4 were solved by the least square method, fitting the model to the data  
306 observed in this study.

307

## 308 **3. Results**

### 309 **3.1. Evolution of humus parameters during incubation**

310 Mean values of  $\text{pH}_{\text{water}}$ , carbon content (%C) and the carbon-to-nitrogen (C/N) ratio of  
311 humus during the incubation period are presented in Table 2. Values for each sampling time  
312 are presented in SI Table S2 and Table S3.

313

314 Table 2. Mean values ( $\pm$  standard error) of  $\text{pH}_{\text{water}}$ , carbon content (%C) and carbon-to-  
 315 nitrogen (C/N) ratio of spiked (R1 and R2) and unspiked (R3 and R4) humus.

		$\text{pH}_{\text{water}}$	%C	C/N ratio
CHP40 (mull)	R1-R2	$6.4 \pm 0.3$	$41.1 \pm 1.6$	$19.4 \pm 1.6$
	R3-R4	$6.3 \pm 0.2$	$39.5 \pm 2.4$	$20.6 \pm 1.9$
CHS35 (moder)	R1-R2	$6.1 \pm 0.9$	$42.7 \pm 1.1$	$16.4 \pm 1.1$
	R3-R4	$6.4 \pm 0.5$	$42.7 \pm 0.6$	$17.7 \pm 0.6$
HET25 (mull)	R1-R2	$6.9 \pm 0.4$	$35.5 \pm 1.6$	$20.0 \pm 1.1$
	R3-R4	$6.4 \pm 0.5$	$32.9 \pm 1.3$	$19.2 \pm 0.9$
HET29 (moder)	R1-R2	$6.2 \pm 0.3$	$44.4 \pm 1.6$	$18.6 \pm 1.6$
	R3-R4	$6.1 \pm 0.3$	$44.1 \pm 0.9$	$20.0 \pm 1.8$
SP25 (mull)	R1-R2	$7.2 \pm 0.4$	$39.3 \pm 1.9$	$24.2 \pm 2.5$
	R3-R4	$6.9 \pm 0.4$	$38.7 \pm 2.0$	$25.7 \pm 1.2$
SP63 (moder)	R1-R2	$5.8 \pm 0.5$	$46.1 \pm 1.2$	$20.7 \pm 1.5$
	R3-R4	$5.4 \pm 0.4$	$45.0 \pm 0.6$	$21.3 \pm 0.8$

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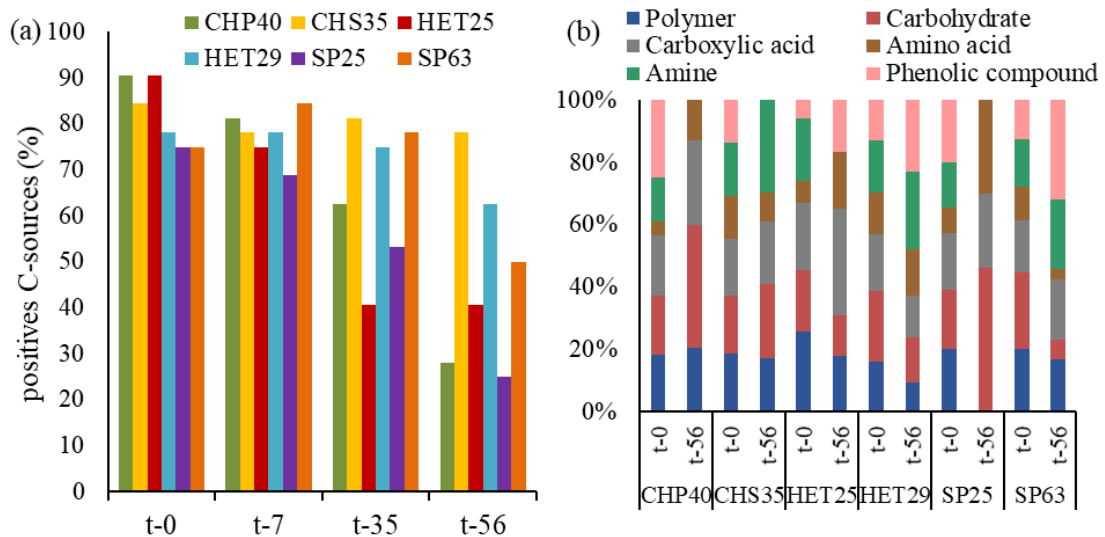
317 On average over the incubation time,  $\text{pH}_{\text{water}}$  showed no significant variation. CHP40,  
 318 CHS35, HET29 and SP63 humus were slightly more acidic ( $\text{pH}_{\text{water}} \sim 5-6$ ) than HET25 and  
 319 SP25 humus ( $\text{pH}_{\text{water}} \sim 7$ ). The carbon content showed no significant variation during incubation  
 320 and ranged from 33 to 46% depending on the humus sample. The C/N ratios were  $\sim 19-26$  for  
 321 mull forms (i.e. CHP40, HET25 and SP25) and  $\sim 16-21$  for moder forms (i.e. CHS35, SP63 and  
 322 HET29), and remained relatively constant across the incubation period. According to  
 323 Duchaufour (1950), OM with a high C/N ratio ( $\sim 15-20$ ) decomposes slowly, and results solely  
 324 in the release of carbon dioxide, with total nitrogen being used for microbial syntheses. For the  
 325 first water-extract (W1), concentrations of dissolved organic carbon (DOC) were higher for  
 326 moder than for mull humus (SI Table S4 and Table S5; on average,  $\sim 360-540$  and  $\sim 150-340$   
 327  $\text{mg L}^{-1}$  for spiked humus;  $\sim 215-300$  and  $\sim 70-210$   $\text{mg L}^{-1}$  for unspiked humus, respectively),  
 328 probably due to slightly higher carbon content ( $\sim 43-46$  and  $36-41\%$ , respectively; Table 2). The  
 329 fluorescence index (FI) in TMAH-extracts was usually lower than in water-extracts, suggesting  
 330 that, as expected, TMAH-extracted organic matter was more aromatic than that solubilized in  
 331 water (SI Figure S3; McKnight et al., 2001). Moreover, FI values showed no clear significant  
 332 evolution over the incubation period. As detailed analysis regarding the composition of residual

333 humus was not carried out, the nature of the molecules constituting the residual fraction is  
334 unclear. However, it can be expected that the residual fraction would be composed of more  
335 complex OM and, therefore, remains undissolved in both water and TMAH. The aromaticity or  
336 molecular weight of the OM could increase depending on the strength of the extractant: w-  
337 soluble < TMAH-available < residual fractions.

338         Community-level physiological profiles (CLPP) assessed with the Biolog EcoPlate™  
339 for spiked humus are presented Figure 2 (a). At t-0, the number of positive C-sources presented  
340 no clear difference between types of humus and accounted for ~70-90% of the sources tested.  
341 However, for CHP40, HET25 and SP25, the number of positive C-sources started to decrease  
342 at t-35 up to t-56 (~20-40% positive C-sources); whereas the number of positive C-sources  
343 slightly decreased up to t-56 (~50-60% positive C-sources) for HET29 and SP63, and remained  
344 constant for CHS35. Hence, in our incubation experiment, microbial activity in mull humus  
345 (i.e. CHP40, HET25 and SP25) decreased more rapidly than in moder humus (i.e. CHS35,  
346 HET29 and SP63). Regarding the chemical nature of the substrates (Figure 2 (b)), at t-0 the six  
347 spiked humus samples used the six guilds of C-source in almost the same proportions. However,  
348 in addition to a decrease in total bacterial activity at t-56, the substrates utilization was different  
349 compared to t-0. Bacteria in the CHP40 and SP25 humus used only 3-4 carbon guilds, and five  
350 carbon guilds in HET25 and CHS35. For HET29 and SP63, the six guilds of C-source were  
351 used throughout the experiments in varying proportions during incubation. No difference in  
352 substrate use depending on their chemical nature was found according to humus form.

353





354

355 Figure 2. (a) Proportion of Biolog EcoPlate™ carbon sources accounted as positives for  
 356 sampling time from t-0 to t-56, and (b) contribution of substrate guilds in total bacteria  
 357 activity at t-0 and t-56 for spiked humus (R1, n = 1).

358

### 359 3.2. Evolution of iodine w-solubility and its organic-pool

360 The evolutions of w-soluble, organic and residual <sup>125</sup>I fractions are shown in Figure 3.

361 The corresponding modelling results obtained from Eq. 3 (see §2.5.) are compiled in SI Table

362 S6. Native w-soluble, organic and residual <sup>127</sup>I fractions at t-0 and at the end of experiment (t-

363 112) for unspiked humus are compared to those of added <sup>125</sup>I in Figure 4. The corresponding

364 detailed data are reported in SI Table S7 and Table S8 for spiked and unspiked humus,

365 respectively.

366 Factoring in uncertainties, total <sup>125</sup>I activities were similar across the incubation period

367 for all forms of humus, with a mean for duplicates and the seven sampling dates of: CHP40 =

368  $37 \pm 2$ , CHS35 =  $42 \pm 3$ , HET25 =  $38 \pm 2$ , HET29 =  $42 \pm 2$ , SP25 =  $37 \pm 2$  and SP63 =  $36 \pm 2$

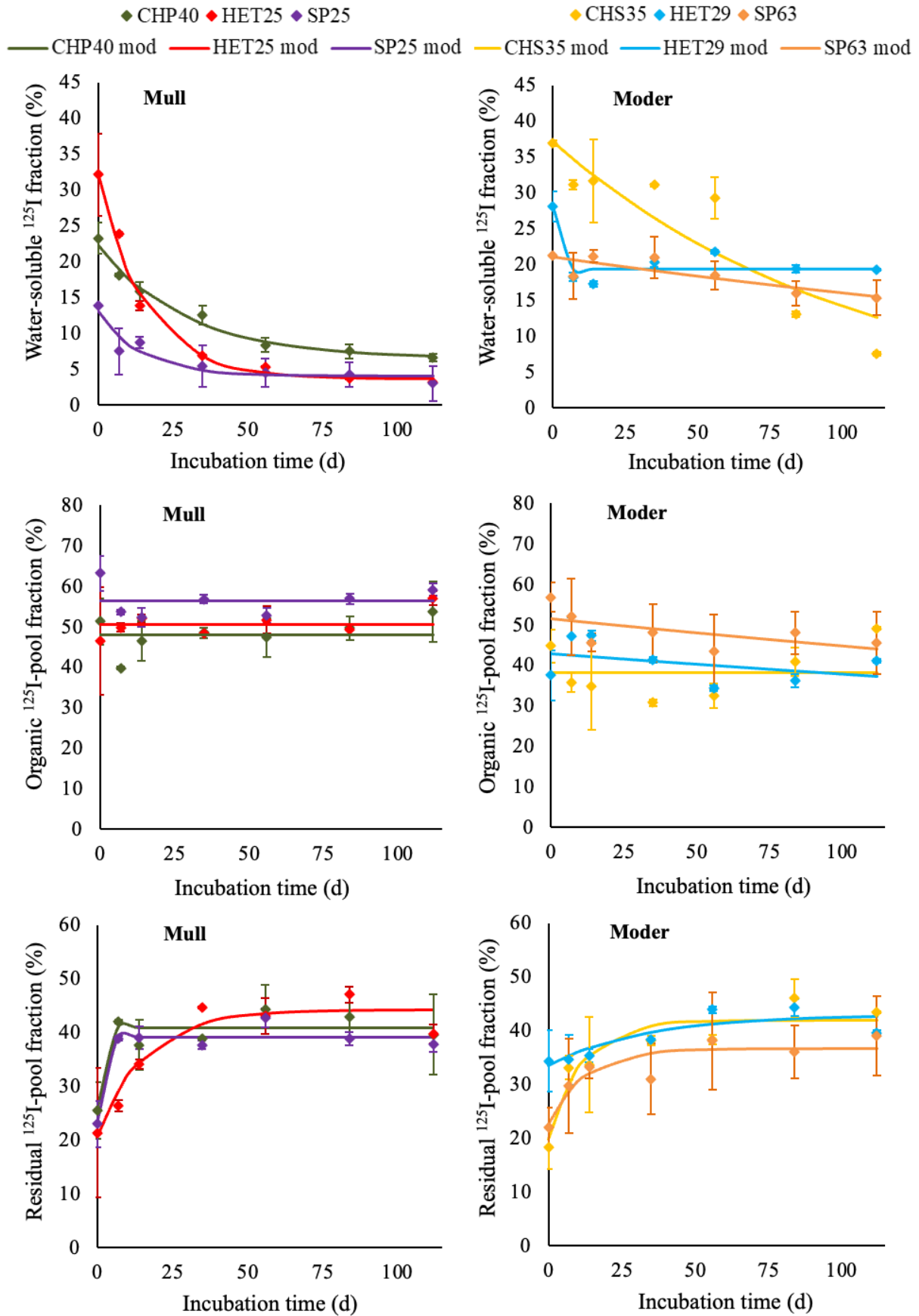
369 kBq g<sup>-1</sup> (SI Figure S4). Total concentrations of stable iodine naturally present in unspiked

370 humus were constant over the incubation period for all forms of humus, ranging from 2.7 to 5.3

371 mg kg<sup>-1</sup> with a mean for duplicates and the two sampling periods of: CHP40 =  $2.7 \pm 0.2$ , CHS35

372 =  $3.5 \pm 0.2$ , HET25 =  $3.1 \pm 0.2$ , HET29 =  $5.3 \pm 0.3$ , SP25 =  $2.9 \pm 0.7$  and SP63 =  $4.7 \pm 0.3$  mg

373 kg<sup>-1</sup>.



374

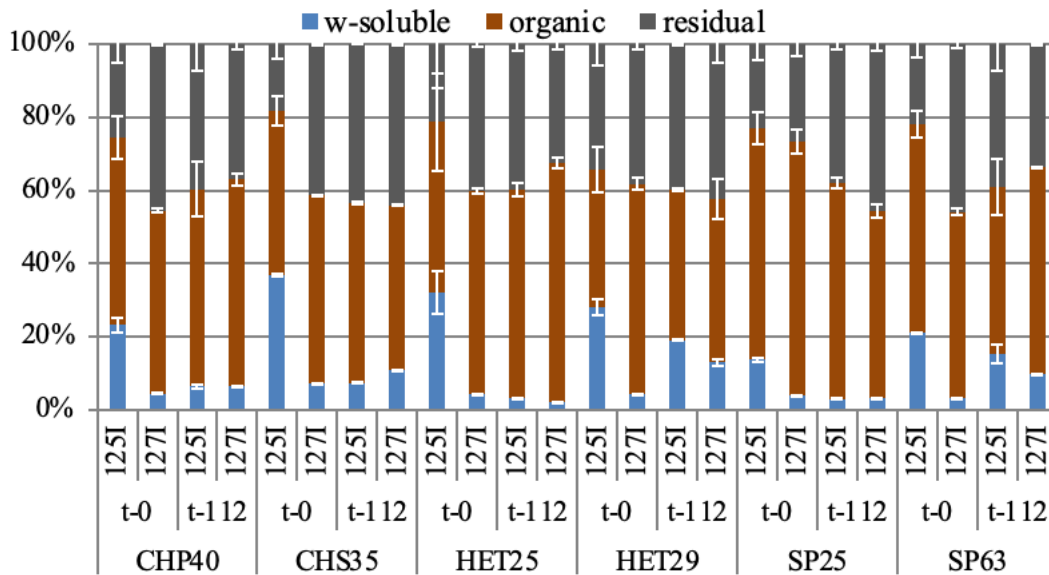
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376

Figure 3. Evolution of w-soluble, organic and residual fractions of added  $^{125}\text{I}$  according to incubation time for mull and moder humus. Dots correspond to experimental mean of

377  
378

duplicates (R1 and R2) with the standard error and lines correspond to the model fitted to experimental data.



379

380 Figure 4. Native w-soluble, organic and residual <sup>127</sup>I fractions at the beginning (t-0) and at the  
381 end (t-112) of experiment for unspiked humus (R3 and R4), compared to those of added <sup>125</sup>I  
382 for spiked humus (R1 and R2). Mean values for duplicates and the standard error.

383

384 Significant differences in <sup>125</sup>I water-solubility were found between the humus samples  
385 with initial (t-0) proportions ranging from ~14 to 37% (CHS35 = 36.9 ± 0.4, HET25 = 32.1 ±  
386 5.8, HET29 = 28.1 ± 2.1, CHP40 = 23.2 ± 2.2, SP63 = 21.2 ± 0.1 and SP25 = 13.8 ± 0.5%). A  
387 significant decrease in <sup>125</sup>I water-solubility occurred over the incubation time for all samples  
388 with different kinetics to reach at t-112: CHS35 = 7.5 ± 0.3, HET25 = 3.2 ± 0.1, HET29 = 19.2  
389 ± 0.1, CHP40 = 6.6 ± 0.6, SP63 = 15.4 ± 2.4 and SP25 = 3.0 ± 0.1% (Figure 3). For mull humus  
390 samples, i.e. CHP40, HET25 and SP25, the w-soluble <sup>125</sup>I fraction decreased within the first 2-  
391 3 months of incubation by a factor of 3.5, 10.0 and 4.6, respectively, with kinetic constants  
392 varying by a factor of 2 ( $k_w \sim 0.034, 0.063$  and  $0.069 \text{ d}^{-1}$ , respectively;  $R^2 > 0.90$ ) and then  
393 remaining quite stable, to reach the lowest values measured. For moder humus, the shape of the  
394 decrease is different to that for mull humus. For HET29 humus, the initial proportion of w-  
395 soluble <sup>125</sup>I (~28%) decreased rapidly (< one week after contamination), and then stabilized  
396 with the highest proportion of w-soluble <sup>125</sup>I (~19%) at the end of incubation of all the humus

397 samples. CHS35 and SP63 humus presented a continuous and slowest decrease of their w-  
398 soluble  $^{125}\text{I}$  fraction ( $k_w \sim 0.010$  and  $0.003 \text{ d}^{-1}$ , respectively;  $R^2 > 0.73$ ) from the beginning to  
399 the end of the study. Last, at t-112,  $^{125}\text{I}$  was less w-soluble in moder humus compared to mull  
400 humus. Regarding the dry mass residence time in humus of 1.1-4.8 and 12.5-16.8 years for mull  
401 and moders, respectively (Table 1), the w-soluble  $^{125}\text{I}$  fraction decreased 18-177 times faster  
402 than the degradation of humus (SI Table S6;  $t_{1/2} = 10-20$  and  $72-258$  days for mull and moder).  
403 These results suggest that rain controls the vertical migration of iodine by leaching in the first  
404 months following iodine deposition; while over a longer term, iodine migration is controlled by  
405 OM degradation. In the case of stable iodine, the w-soluble  $^{127}\text{I}$  fraction was very low compared  
406 to the values obtained for  $^{125}\text{I}$  at the beginning of the experiments (by factors of from 3 to 7)  
407 and evolved very little from t-0 to t-112 (factor  $\approx 1.5$ ) except for HET 29 and SP63 moder  
408 humus, in which the w-soluble  $^{127}\text{I}$  fraction increased by a factor 3, while for HET25 mull  
409 humus, it was reduced by half (SI Table S8). At the end of the incubation period, w-soluble  $^{127}\text{I}$   
410 fractions in unspiked humus accounted for: CHP40 =  $6.64 \pm 0.01$ , CHS35 =  $11.04 \pm 0.06$ ,  
411 HET25 =  $2.21 \pm 0.01$ , HET29 =  $13 \pm 1$ , SP25 =  $3.32 \pm 0.03$  and SP63 =  $9.72 \pm 0.02\%$ . After 4  
412 months, w-soluble proportions of added  $^{125}\text{I}$  were relatively similar to the results obtained for  
413 natural  $^{127}\text{I}$  (Figure 4), implying that added  $^{125}\text{I}$  and native iodine tended towards the same  
414 distribution. Furthermore, the nature of the humus appears to be a factor influencing the level  
415 of  $^{125}\text{I}$  water-solubility and its evolution over time. Indeed, a rapid and large decrease in  $^{125}\text{I}$   
416 solubility occurred for mull humus (i.e. CHP40, HET25 and SP25), with the w-soluble fraction  
417 decreasing from  $\sim 14-32$  to  $3-7\%$ ; whereas for moder humus (i.e. CHS35, HET29 and SP63),  
418 the decrease in  $^{125}\text{I}$  solubility was slower and smaller (from  $\sim 21-37$  to  $7-19\%$ ) (Figure 3). To  
419 our knowledge, this is the first study of iodine-humus interaction, so direct comparison with  
420 data in the literature is therefore difficult. However, our results obtained regarding the evolution  
421 of iodine w-solubility and its proportion at the end of incubation for humus agree with results

422 previously obtained for soil (Ashworth et al., 2003; Bostock et al., 2003; Hansen et al., 2011;  
423 Hou et al., 2003; Qiao et al., 2012; Takeda et al., 2015). All these authors, regardless of the type  
424 of iodine addition (stable or radioactive, iodide or iodate) in soil, observed a decrease in its w-  
425 soluble fraction over time. More precisely, for forest soils, Bostock et al. (2003) and Takeda et  
426 al. (2015) quantified a decrease in w-soluble spiked iodine fraction from > 50% to < 7% after  
427 around one month of incubation, which they attributed to rapid conversion of added inorganic  
428 iodine to insoluble organic iodine.

429 The TMAH-extraction showed that a high  $^{125}\text{I}$  fraction was immediately associated with  
430 the organic pool with initial proportions of:  $\text{CHP40} = 51 \pm 6$ ,  $\text{CHS35} = 45 \pm 4$ ,  $\text{HET25} = 46 \pm$   
431  $13$ ,  $\text{HET29} = 37 \pm 6$ ,  $\text{SP25} = 63 \pm 4$  and  $\text{SP63} = 57 \pm 4\%$  (Figure 3). Surprisingly, factoring in  
432 uncertainties, the proportions of organic  $^{125}\text{I}$  did not change clearly during incubation, except  
433 in case of CHS35. For this humus, the organic  $^{125}\text{I}$  fraction decreased to 31% up to t-35 and  
434 then increased to ~49% up to t-112. Mull humus (i.e. CHP40, HET25 and SP25) presented  
435 slightly higher organic  $^{125}\text{I}$  fractions than moder humus (i.e. CHS35, HET29 and SP63): on  
436 average ~57 and 45% at t-112, respectively (Figure 3). For native iodine in unspiked humus,  
437 organic  $^{127}\text{I}$  fractions evolved only slightly from t-0 to t-112 (a factor of 1.1-1.4; SI Table S8),  
438 and at the end of incubation that fraction accounted for an average of ~ 58 and 49% for mull  
439 and moder humus, respectively. Iodine-127 was therefore mostly organically bound in most  
440 samples (~45-65%) and, as observed for the w-soluble fraction, proportions of added  $^{125}\text{I}$   
441 measured in the organic fraction were close to native iodine distribution at the end of incubation  
442 (Figure 4). Similarly, Hansen et al. (2011) found that humic substances extracted with 5%  
443 TMAH contained about 49-60% of total  $^{127}\text{I}$  and  $^{129}\text{I}$  for a composite of Danish soils. In Qiao  
444 et al. (2012),  $^{129}\text{I}$  and  $^{127}\text{I}$  were mainly distributed in the OM-related fraction (38 and 55%,  
445 respectively). Ashworth et al. (2003) found that NaOH extracted on average 60% of added  $^{125}\text{I}$   
446 in soil. In Bostock et al. (2003),  $^{125}\text{I}^-$  has been shown to associate mostly with humic substances

447 within the soil with 87% of added iodine extracted by NaOH. However, these authors indicated  
448 that this percentage was overestimated because the w-soluble  $^{125}\text{I}$  fraction was included in this  
449 fraction, which is in line with our results. Xu et al. (2011b) demonstrated that humic substances  
450 accounted for around 55 and 46% of the total  $^{127}\text{I}$  and  $^{129}\text{I}$  in soils, respectively.

451 In our study, the initial proportions of residual  $^{125}\text{I}$ -pool were relatively similar  
452 regardless of humus type (~19-26%), except for HET29 showing a slightly higher proportion  
453 (CHP40 =  $25 \pm 5$ , CHS35 =  $18 \pm 4$ , HET25 =  $21 \pm 12$ , HET29 =  $34 \pm 6$ , SP25 =  $23 \pm 4$  and  
454 SP63 =  $22 \pm 4\%$ ; Figure 3). The proportions of residual  $^{125}\text{I}$  increased within the first month of  
455 incubation with different kinetics to reach at t-112: CHP40 =  $40 \pm 7$ , CHS35 =  $43.4 \pm 0.2$ ,  
456 HET25 =  $40 \pm 2$ , HET29 =  $39.7 \pm 0.4$ , SP25 =  $38 \pm 1$  and SP63 =  $39 \pm 7\%$ . The humus CHP40  
457 and SP25 presented the fastest increase (for CHP40, stabilization after one week; for SP25,  $k_r$   
458  $\sim 0.56 \text{ d}^{-1}$ ;  $R^2 = 0.93$ ) with initial proportion of residual  $^{125}\text{I}$  multiplied by a factor of  $\sim 1.5$  after  
459 only 14 days and remaining constant throughout the rest of incubation period. For the CHS35,  
460 HET25 and SP63 humus, initial proportions increased by a factor of  $\sim 2$  to reach steady values  
461 after  $<$  two months ( $k_r \sim 0.09, 0.06$  and  $0.08 \text{ d}^{-1}$ ;  $R^2 = 0.89, 0.91$  and  $0.84$ ; respectively). Last,  
462 the HET29 humus, whose initial residual fraction was the highest, presented the slowest and  
463 smallest increase ( $k_r \sim 0.03 \text{ d}^{-1}$ ,  $R^2 = 0.74$ ). A relatively large part of  $^{125}\text{I}$  ( $\sim 40\%$ ) was therefore  
464 found in the residue not available with TMAH or UHQ water at room temperature. The same  
465 observation was made for native iodine in unspiked humus with residual proportion in the same  
466 order of magnitude ( $\sim 33\text{-}46\%$ ) (Figure 4). Overall, the same evolution of  $^{125}\text{I}$  was observed for  
467 all types of humus, with faster stabilization for CHP40 and SP25 and less amplitude and weaker  
468 stabilization for HET29 (Figure 3).

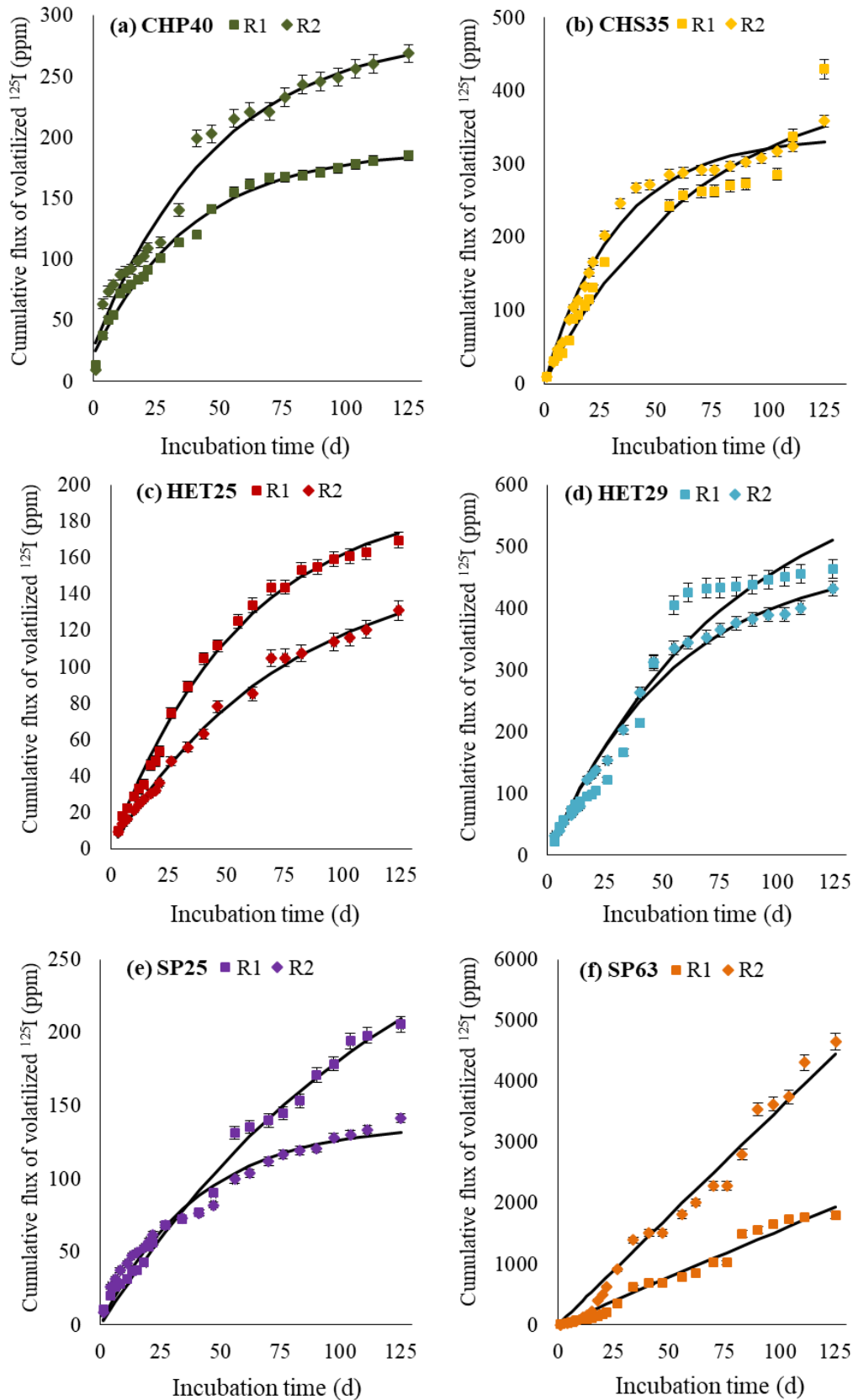
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### 470 3.3. $^{125}\text{I}$ volatilization from humus

471  $86 \pm 21\%$  of total volatilized  $^{125}\text{I}$  was trapped in the first activated carbon trap, whereas  
472 only  $14 \pm 21\%$  was trapped in the second trap. The evolution of  $^{125}\text{I}$  volatilization rates over the  
473 humus incubation period is shown in SI Figure S5. Volatilization rates varied over the 125 days  
474 of monitoring, with extreme values ranging within three orders of magnitude (CHP40 =  
475  $5.7 \times 10^{-8} - 1.6 \times 10^{-5}$ , CHS35 =  $2.6 \times 10^{-8} - 1.3 \times 10^{-5}$ , HET25 =  $3.6 \times 10^{-8} - 0.4 \times 10^{-5}$ , HET29 =  
476  $7.0 \times 10^{-8} - 1.5 \times 10^{-5}$ , SP25 =  $0.3 \times 10^{-8} - 0.9 \times 10^{-5}$ , SP63 =  $6.4 \times 10^{-8} - 8.7 \times 10^{-5} \text{ day}^{-1}$ ).  
477 Generally, initial  $^{125}\text{I}$  volatilization rates declined over the experiment time, except in the case  
478 of SP63. This humus differed from the others since the  $^{125}\text{I}$  volatilization rate increased over  
479 the first month, while no specific trend was seen in the following months. Moreover,  $^{125}\text{I}$   
480 volatilization rates from SP63 humus were higher than those for the other humus samples (by  
481 a factor of from 6 to 35), if maximum values are compared. The mean  $^{125}\text{I}$  volatilization rates,  
482 estimated by the modelling of the cumulative fluxes of volatilized  $^{125}\text{I}$  using Eq. 4 (SI Table S9;  
483 see §2.5.), were: CHP40 =  $1.8 \pm 0.3 \times 10^{-6}$ , CHS35 =  $3.0 \pm 0.4 \times 10^{-6}$ , HET25 =  $1.2 \pm 0.2 \times 10^{-6}$ ,  
484 HET29 =  $3.8 \pm 0.3 \times 10^{-6}$ , SP25 =  $1.4 \pm 0.3 \times 10^{-6}$ , SP63 =  $26 \pm 10 \times 10^{-6} \text{ day}^{-1}$ , confirming  
485 that volatilization from the SP63 humus was the highest. Regarding cumulative iodine fluxes,  
486 a significant increase could be observed during the first two months of incubation, followed by  
487 lesser augmentation for all spiked samples, except for SP63 whose cumulative fluxes increased  
488 linearly with time (Figure 5). Although in the same order of magnitude, differences in total  
489 volatilized  $^{125}\text{I}$  over the incubation periods were observed according to humus type. The total  
490 amount of volatile iodine appeared slightly higher for moder humus ( $0.039 \pm 0.004$ ,  $0.045 \pm$   
491  $0.002$  and  $0.32 \pm 0.14\%$  for CHS35, HET29 and SP63, respectively) than for mull humus ( $0.023$   
492  $\pm 0.004$ ,  $0.015 \pm 0.002$  and  $0.017 \pm 0.003\%$  for CHP40, HET25 and SP25, respectively). These  
493 amounts were relatively similar between duplicates (RSD = 5-26%), except in the case of SP63  
494 (RSD = 62%). For all types of humus studied,  $^{125}\text{I}$  volatilization rates were within the range of  
495 emission rates found in the literature for soils (Bostock et al., 2003; Muramatsu and Yoshida,

496 1995; Wildung et al., 1985). In Bostock et al. (2003), over 22 days incubation,  $^{125}\text{I}$  volatilization  
497 rates from forest soil ranged between  $6 \times 10^{-7}$  and  $5 \times 10^{-5} \text{ day}^{-1}$ , and initially declined steeply  
498 followed by a subsequent slower decline. After adding  $^{129}\text{I}$  to soil, Wildung et al. (1985) found  
499 an averaged volatilization rate of  $2 \times 10^{-5} \text{ day}^{-1}$  from soil over 14 days. Muramatsu and Yoshida  
500 (1995) found a rate of volatilization ranging between  $0.4 \times 10^{-5}$  to  $4 \times 10^{-5} \text{ day}^{-1}$  over 80 days  
501 after adding  $^{129}\text{I}^-$  addition onto unplanted non-flooded soil.





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Figure 5. Cumulative flux of volatilized  $^{125}\text{I}$  over the 125 days monitoring period for each humus (mull: (a) (c) (e), moder: (b) (d) (f)). R1 and R2 correspond to duplicates. Dots correspond to experimental values with standard deviation and lines correspond to model fitted to experimental data.

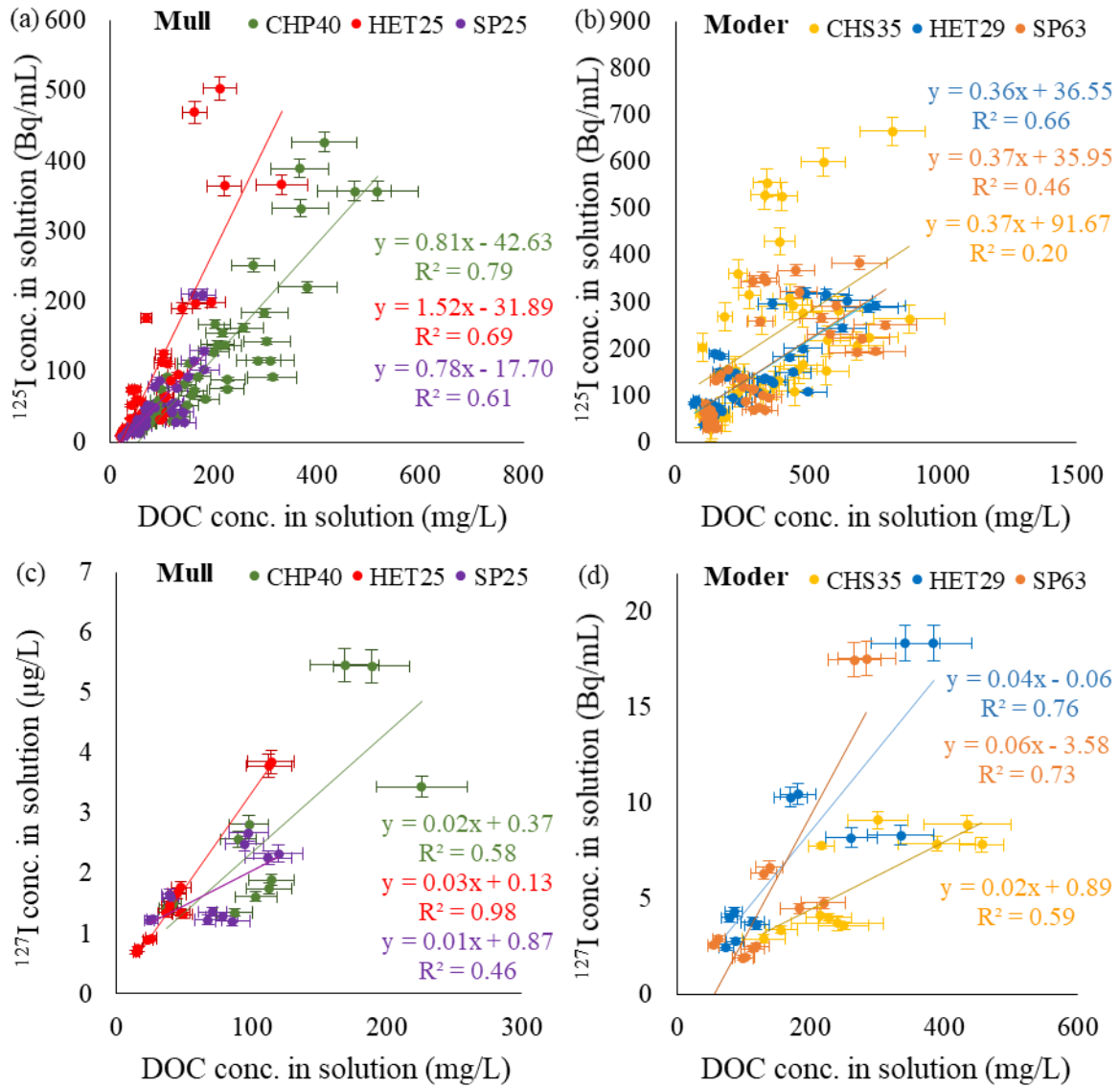
#### 507 4. Discussion

508 Results from the current study suggest that humus is a zone of iodine accumulation by  
509 association with OM (organic and residual  $^{125}\text{I}$  fractions = 37-63 and 38-43%, respectively) and  
510 that potential  $^{125}\text{I}$  losses by lixiviation are far more significant than those by volatilization (w-  
511 soluble and volatilized  $^{125}\text{I}$  fractions = 3-19% and 0.01-0.47%, respectively).

512 It appeared that added  $^{125}\text{I}$  was progressively more strongly linked to humus  
513 components, depleting the w-solubility of iodine and resulting in a distribution similar to that  
514 of native iodine at the end of incubation (Figure 4). This iodination seemed to evolve differently  
515 according to humus form. Indeed, the composition of moder humus, which probably contains  
516 more complex organic molecules (slow degradation) than mulls (fast degradation) would lead  
517 to slower iodination of complex organic molecules and therefore higher w-solubility of  $^{125}\text{I}$  in  
518 moders than mulls (Figure 3). This would be consistent with previous studies showing that  
519 lowest molecular weight (LMW) humic substances react more easily with iodine due to greater  
520 surface area which may provide greater accessibility to reactive sites, unlike high molecular  
521 weight (HMW) humic substances, whose iodination could be time-dependent (Bowley et al.,  
522 2016; Xu et al., 2011a, 2011b). Moreover, in our study, moder humus showed a slight increase  
523 in the w-soluble  $^{127}\text{I}$  fraction (Figure 4) suggesting a deiodination process and thus a weaker  
524 affinity of iodine with moder than mull humus, which is consistent with a weaker stabilization  
525 of the w-soluble  $^{125}\text{I}$  fraction for HET29 and SP63 (Figure 3). Furthermore,  $^{125}\text{I}$  and  $^{127}\text{I}$  w-  
526 soluble concentrations were positively correlated with DOC concentrations in water-extracts  
527 (Figure 6), which were higher for moders than mulls (SI Table S4 and Table S5) leading to  
528 increased quantities of w-soluble  $^{125}\text{I}$  and  $^{127}\text{I}$  linked to dissolved OM. After water extraction of  
529 soils from the Savannah River site contaminated by  $^{129}\text{I}$ , Xu et al. (2011b) indicated that some  
530 colloidal and dissolved organic matter was either iodinated by radioiodine in soil solution or  
531 co-released from solids containing radioiodine previously incorporated into the soil. During

532 batch sorption experiments of iodine in humus, Söderlund et al. (2017) also found an  
533 unidentified iodine species in the liquid phase analyzed using HPLC-ICP-MS, thus supporting  
534 the hypothesis of OM-I association in solution. More recently, Takeda et al. (2019) showed that  
535 the concentration of dissolved organic iodine, obtained by subtracting the  $I^-$  and  $IO_3^-$   
536 concentrations from the total I concentration, was positively correlated with the DOC of the  
537 soil solution extracted by centrifugation from Japanese forest soils. Likewise, Xu et al. (2011a)  
538 showed that w-soluble  $^{129}I$  in soil was mostly associated with a low molecular weight organic  
539 carrier (13.5–15 kDa). Hence, our results would indicate a dual role played by organic matter:  
540 (1) humic substances of HMW limiting DOC would be iodine sinks and (2) humic substances  
541 of LMW contributing to DOC would be a source for subsurface iodine dispersion, as previously  
542 observed by Chang et al. (2014) and Unno et al. (2017).

543



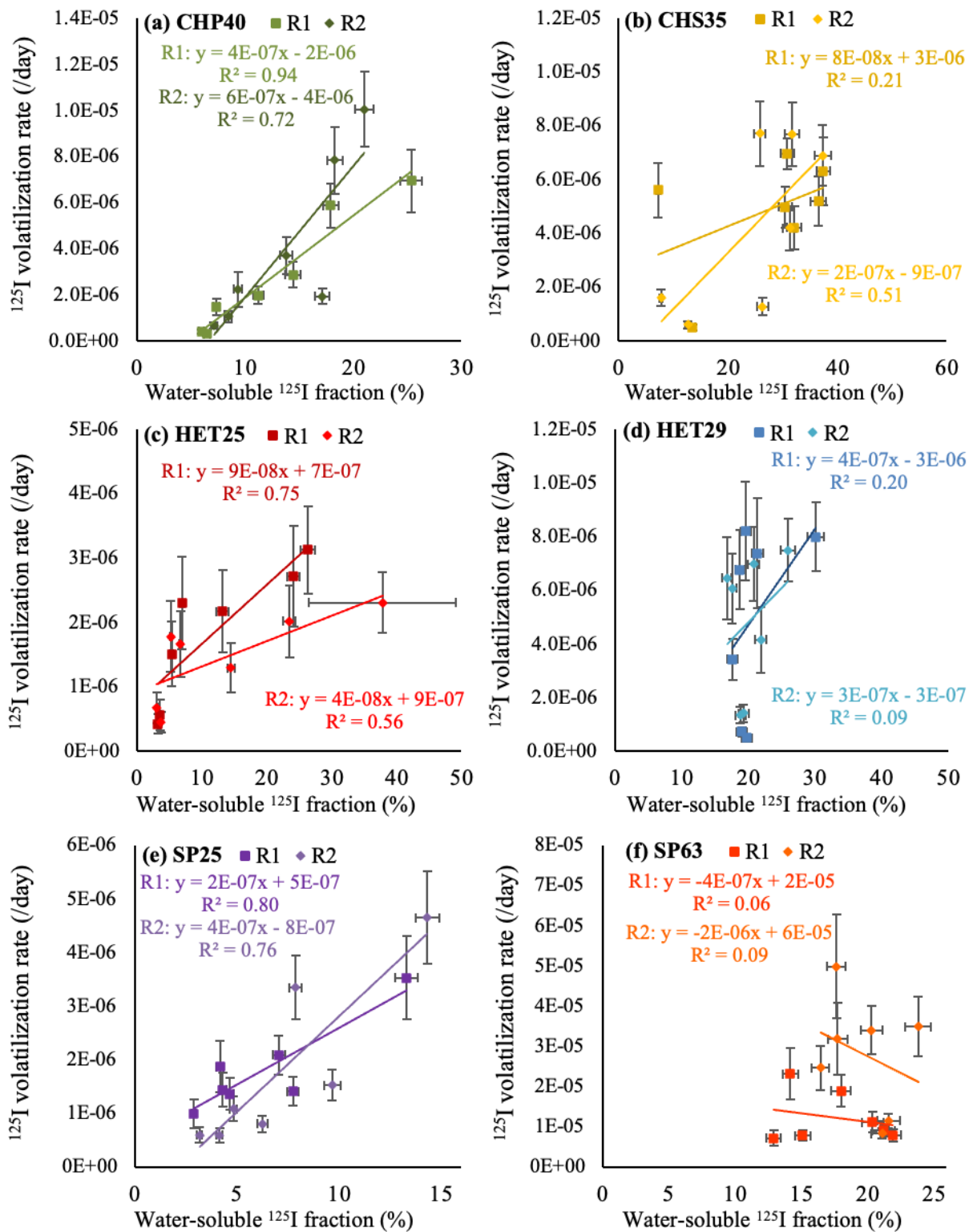
544

545 Figure 6.  $^{125}\text{I}$  and  $^{127}\text{I}$  concentrations in w-soluble extracts as a function of concentrations of  
 546 dissolved organic carbon for the w-soluble fraction in mull and moder humus. Values ( $\pm$   
 547 standard error) for the duplicates and all sampling points.

548

549 Bostock et al. (2003) suggested that iodine association with OM occurred as a two-stage  
 550 process according to their  $^{125}\text{I}$  emission rates declining over time, initially rapid and then much  
 551 slower. In the current study, the iodination process results in a reduction of the w-soluble iodine  
 552 pool which might induce an effective reduction of the volatilization. This assumption is  
 553 supported by the positive correlation between  $^{125}\text{I}$  volatilization rate and w-soluble fraction for  
 554 mulls (i.e. CHP40, HET25 and SP25; Figure 7 (a)(c)(e),  $R^2 > 0.56$ ). However, no correlation

555 was found for moders (i.e. CHS35, HET29 and SP63; Figure 7 (b)(d)(f)), involving another  
556 source of  $^{125}\text{I}$  volatilization for this humus form in place of or additionally to the quantity of  
557 iodine soluble in solution. As previously shown, the values for total volatilized  $^{125}\text{I}$  activity were  
558 higher for moder (~0.039-0.323%) than for mull (0.015-0.023%) humus, suggesting that  $^{125}\text{I}$   
559 volatilization could occur directly from the solid phase of humus after its iodination. Iodide  
560 could have been oxidised to reactive  $\text{I}_2$  by reaction with hydroquinone or semiquinone moieties  
561 of organic matter, as proposed by Steinberg et al. (2008a). Allard and Gallard (2013) also  
562 proposed also a mechanism of abiotic formation of iodomethane from pyruvate, an organic  
563 compound derived from the oxidation of humic substances. Thus, differences in the nature of  
564 the humus OM according to forms of humus may also affect  $^{125}\text{I}$  volatilization. Furthermore, it  
565 has been shown that the iodine volatilization process is stimulated by biological activity in the  
566 soil, by external enzymes excreted by microorganisms and/or by a wide variety of bacteria  
567 (Allard et al., 2010; Amachi, 2008; Muramatsu et al., 2004; Muramatsu and Yoshida, 1995).  
568 Amachi et al. (2003) showed that soil bacteria are able to methylate iodine (producing  $\text{CH}_3\text{I}$ ).  
569 Ban-nai et al. (2006), using laboratory cultures, found that filamentous fungi in soils  
570 accumulated and volatilized  $^{125}\text{I}$  (to 3.4% of total I in soil) in  $\text{CH}_3\text{I}$  form. Shimamoto et al.  
571 (2011) and Seki et al. (2013) proposed that oxidase laccase enzyme, produced by soil fungi and  
572 bacteria, is able to oxidize  $\text{I}^-$  into  $\text{I}_2$ . Although these authors suggest that  $\text{I}_2$  reacts with OM to  
573 produce organo-iodinated compounds retained in soil, it is likely that part of  $\text{I}_2$  is directly  
574 volatilized and that some organo-iodinated compounds are converted to  $\text{CH}_3\text{I}$  and then  
575 volatilized. In our study, this assumption is supported by the community-level physiological  
576 profiles (Figure 2) of moders (i.e. CHS35, HET29 and SP63), which decreased more slowly  
577 over time than those of mulls (i.e. CHP40, HET25 and SP25), retaining positive C-sources  
578 >50% up to t-56, that may maintain a higher volatilization rate throughout our experiment than  
579 for mull humus.



580

581 Figure 7. Mean ( $\pm$  standard error) of  $^{125}\text{I}$  volatilization rate by sampling time as a function of  
 582 w-soluble  $^{125}\text{I}$  fraction according to humus sample. For these graphs, values of  $^{125}\text{I}$  volatilized  
 583 fraction correspond to mean of  $^{125}\text{I}$  volatilization rates at some days before and after sampling  
 584 time for w-soluble extraction.

585

586 **Conclusion**

587 Our results show that humus is a zone of iodine accumulation by association with the  
588 solid phase. The w-soluble  $^{125}\text{I}$  fraction decreased over time from ~14-37% to 3-19% depending  
589 on the type of humus. As the organic iodine fraction remained stable (~38-56%), an increase in  
590 residual  $^{125}\text{I}$  was observed (from ~18-34% to 38-43). After 4 months, the proportions of w-  
591 soluble, organic and residual added  $^{125}\text{I}$  were relatively similar to results obtained for natural  
592  $^{127}\text{I}$ , implying that added  $^{125}\text{I}$  tended towards native iodine distribution after a few months. The  
593 more complex nature of organic material in moders (slow degradation) than in mulls (fast  
594 degradation) resulted in slower iodination of organic molecules and thus higher proportions of  
595 w-soluble  $^{125}\text{I}$  for moders than mulls. Significant positive correlations between  $^{125}\text{I}$   
596 concentrations and concentrations of dissolved organic carbon in the w-soluble fraction may  
597 indicate that organic matter plays a dual role, acting both as iodine sink, probably through the  
598 migration of humic substances of high molecular weight, and also as source of subsurface  
599 iodine dispersion, probably through humic substances of low molecular weight. This study  
600 confirms that  $^{125}\text{I}$  losses by volatilization are relatively low (total volatilized  $^{125}\text{I}$  fractions =  
601 0.01-0.47%). Although of the same order of magnitude, total  $^{125}\text{I}$  volatilization was higher for  
602 moders (~0.039-0.323%) than for mulls (~0.015-0.023%) for which volatilization rates were  
603 positively correlated to w-soluble iodine fractions during incubation.

604

## 605 **Acknowledgments**

606 The authors would like to acknowledge the technical staff of RENECOFOR – ONF  
607 (Caroline BRUYERE, Geoffroy PERALS, Mauryne VINCENT, Henri JUIF, Didier  
608 LAGREDE and Yves LE VALLEGANT) for logistical support for the sampling of selected  
609 humus. We would like to acknowledge Anne-Cécile GREGOIRE (IRSN/PSN-  
610 RES/SEREX/L2EC) for advices and part of incubation material. This work was financed by the  
611 Agence Nationale de la Recherche with funds allocated in the ‘Investissements d’Avenir’

612 framework program under reference ANR11-RSNR-0002 and the Région Nouvelle Aquitaine.  
613 We would like to acknowledge the translation company CAUPENNE & CO for English  
614 language assistance.

615

### 616 **Supplementary information**

617 A map of the localisation of the six sampling sites. Figures of evolution of (i) the water  
618 contents, (ii) the fluorescence index measured in water-extracts and TMAH-extracts, (iii) the  
619 total  $^{125}\text{I}$  activity, and (iv) the  $^{125}\text{I}$  volatilization rates from spiked humus during incubation time.

620 A table with the characteristics of the carbon substrates in Biolog EcoPlate™ and chemical  
621 guilds. Tables of the detailed dataset of humus parameters (i.e. pH, C content, C/N ratio, DOC  
622 concentrations) and  $^{127}\text{I}$  and  $^{129}\text{I}$  proportions in water- and TMAH-extracts. Tables of the  
623 modelling results of the evolutions of  $^{125}\text{I}$  w-soluble, organic and residual fractions, and of the  
624  $^{125}\text{I}$  volatilization rates and cumulative fluxes.

625



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