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1 **Early degradation of plant alkanes in soils:**

2 **A litterbag experiment using <sup>13</sup>C-labelled leaves**

3

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19

20 **Highlights:**

- 21
- 22 • specific isotope composition of long-chain alkanes was modified during leaf  
23 decay
  - 24 • isotope modification during leaf decay probably reflects a microbial  
25 contribution to leaf alkanes
  - 26 • long-chain alkanes may not be used as an unaltered record of plant isotope  
27 composition
- 28

29 **Abstract**

30

31 We monitored the carbon isotope composition of bulk leaves and specific long-  
32 chain alkanes during a four-year litterbag experiment using  $^{13}\text{C}$ -labelled leaves and  
33 unlabelled reference leaves of the European beech tree (*Fagus sylvatica* L.).  
34 Whereas the isotope composition of alkanes from  $^{13}\text{C}$ -enriched leaves exhibited a  
35 marked decrease in  $^{13}\text{C}$ -content, the isotope composition of unlabelled reference  
36 leaves remained nearly constant. We interpreted this difference as evidence for a  
37 microbial contribution to the long-chain alkane pool of the decomposing leaves and  
38 related it to the progressive invasion of leaves by soil organisms which was revealed  
39 upon microscopic examination. These results suggest that long-chain alkanes may  
40 not provide an unaltered record of organic carbon isotope composition in soils and  
41 sediments.

42

43 *Keywords: leaf litter decomposition, stable carbon isotopes, lipids, soil organic*  
44 *matter, n-alkanes*

45

46

47 **1. Introduction**

48

49 The carbon isotope composition of organic matter in soils and sediments has  
50 proven useful in documenting past environments, carbon dynamics and the sources  
51 of organic matter. However, the isotope composition of bulk organic matter may be  
52 altered during decomposition and/or by diagenesis. Indeed, the isotope composition  
53 of bulk organic matter corresponds to the weighted mean comprised by the isotope  
54 composition of each of its individual constituents. Therefore, the isotope composition  
55 of bulk organic matter can be disproportionately altered if the constituents lost by  
56 degradation have an isotope composition which differs substantially from that of the  
57 preserved constituents (Deines, 1980; Balesdent et al., 1993). Consequently, the  
58 isotope analysis of specific compounds having high preservation potential may  
59 minimize degradation biases and is becoming increasingly favoured (Wiesenberg et  
60 al., 2004; Yamamoto et al., 2010). Among such biomarkers, long-chain fatty lipids  
61 such as *n*-alkanes and *n*-acids are the most commonly investigated molecules for  
62 specific compound isotope analyses. Indeed, these typical plant components exhibit

63 a rather high preservation potential and are relatively easy to analyse. However,  
64 these long-chain lipids may not be completely resistant to degradation and their  
65 relative distribution may be modified in the early phases of organic matter  
66 degradation (Cranwell, 1981; Buggle et al., 2010). Previous investigations on the  
67 effects of degradation on the isotope composition of these specific lipids led to  
68 contrasting results: either the  $^{13}\text{C}$ -content of these compounds was unaffected over  
69 time or it exhibited a trend towards  $^{13}\text{C}$ -enrichment of specific molecules (Stahl, 1980;  
70 Huang et al., 1997; Mansuy et al., 1997; Mazéas et al., 2002; Nguyen Tu et al., 2004;  
71 Chikaraishi and Naraoka, 2006). The observed  $^{13}\text{C}$ -enrichment of fatty lipids was  
72 suggested to have two possible causes: (i) an isotope discrimination effect had  
73 occurred during degradation and/or (ii) the fatty lipids were contaminated by long-  
74 chain lipids derived from microbes. In the absence of categorical evidence, neither of  
75 these two alternatives could be favoured or excluded by the previous authors  
76 (Nguyen Tu et al., 2004; Chikaraishi and Naraoka, 2006). Nevertheless, the disparity  
77 in the reported  $^{13}\text{C}$ -content trends is likely to be related to differences in the  
78 environments investigated: i.e., oxic vs. anoxic, well-drained vs. stagnant water, soil  
79 organic matter vs. oil spills, etc. Such a disparity calls for complementary studies in  
80 other environments, to better constrain the potential for bias in the isotope record of  
81 (paleo)environments.

82 The present study aimed to determine the precise effects of early degradation  
83 on the isotope composition of specific alkanes. A major challenge to investigating the  
84 degradation of organic matter is the multiplicity and diversity of its sources. Indeed, it  
85 can be difficult to distinguish the actual effects of decomposition from those related to  
86 variations in the relative contributions of its sources. An alternative approach is to  
87 study materials that are precisely identified, such as the plant leaves that provide the  
88 main source of long-chain alkanes in soils and sediments (Eglinton and Hamilton,  
89 1967). Litterbag experiments constitute a prime technique to monitor the fate of  
90 leaves through litter decomposition. Indeed, this method allows plant debris to be  
91 sampled at specific ages, even at high levels of decomposition (Bocock and Gilbert,  
92 1957; Smith and Bradford, 2003). The present study was thus based on a decay  
93 experiment in which beech leaves were enclosed in litterbags. European beech  
94 (*Fagus sylvatica* L.) was chosen as a source because it is a common inhabitant of  
95 temperate forests.

96 Monitoring the fate of  $^{13}\text{C}$ -labelled molecules has proven useful to unravel  
97 biogeochemical processes in ecological studies (Crossman et al., 2005; Bahri et al.,  
98 2008). Therefore, our experiment was carried out using  $^{13}\text{C}$ -labelled leaves in order  
99 to trace the original plant alkanes and to test for possible contamination by microbial  
100 alkanes. Stable isotope labelling of plants generally led to  $^{13}\text{C}$ -enrichment in the order  
101 of several hundred-fold per mil (Angers et al., 1997; Zeller et al., 2000; Bahri et al.,  
102 2008). However, it may be technically difficult to measure small isotope effects when  
103 dealing with such intense labelling. Limited labelling (i.e. lower than 50‰) provides  
104 the opportunity to detect even minor microbial contribution. We thus used slightly  $^{13}\text{C}$ -  
105 enriched beech leaves to monitor the effects of decomposition during a four-year  
106 experiment in natural forest soil. To our knowledge, this study represents the first  
107 time  $^{13}\text{C}$ -labelled leaves have been used to investigate, in the field, the effects of  
108 degradation on the isotope composition of specific lipids. Our data provide a better  
109 understanding of the sources of potential bias related to degradation effects, and  
110 hence will allow specific compound isotope analysis in (paleo)environmental studies  
111 to be used with greater accuracy and significance.

112  
113

## 114 **2. Material and methods**

115

### 116 *2.1. $^{13}\text{C}$ -labelling of beech leaves*

117

118 Beech seeds were germinated at the end of May 2004. Young seedlings were  
119 transferred at the cotyledon stage to a controlled chamber at the beginning of June  
120 2004. In order to minimize isotope effects linked to soil respiration, plants were grown  
121 on vermiculite and fed three times per week with a previously described nutritional  
122 solution (Coïc and Lesaint, 1983). The  $\text{pCO}_2$  and  $\delta^{13}\text{C}_{\text{atm}}$  were maintained at 380  
123 ppmv and +40‰, respectively. Climate conditions in the chamber were held constant  
124 during the five months of labelling: 70% relative humidity and 20°C day temperature.  
125 Four Osram HQI/D Daylight lamps (400 W/D) provided a light intensity of  
126 approximately  $350 \mu\text{mol}/\text{m}^2 \cdot \text{s}$  of photosynthetically active radiation during a 15 hr  
127 period per day (i.e. 7h-20h). The chamber is described in detail elsewhere (Girardin  
128 et al., 2009). Three weeks before the end of the experiment, the conditions were  
129 modified in order to induce leaf senescence. The temperature was reduced to 18°C,

130 the daylight range was reduced to 8 hr (9h-17h) and nutritional solution was provided  
131 only once per week. The leaves thus obtained exhibited a mean  $\delta^{13}\text{C}$  value of  
132 +25.0‰ and ranged between +16.3‰ and +35.3‰.

133

## 134 2.2. Degradation experiment

135

### 136 2.2.1. Experimental site

137 The degradation experiment was established in the "Breuil-Chenué"  
138 experimental forest site located in Burgundy, France (47°18'10"N, 4°4'44"E). This  
139 forest is part of the "Parc Naturel Régional du Morvan", a protected area where urban  
140 pollution is minimal. The field is situated on a shelf at 650 m above sea level and  
141 exhibits a slight northwest-facing slope. Mean annual temperature and precipitation  
142 at the site are 9°C and 1,280 mm, respectively. The bedrock is the alkaline granite of  
143 "La Pierre qui Vire" covered by a thin layer of eolian silt. Soils are classified as dystric  
144 cambisol (WRB, 2006) with a thick moder on top (~10 cm). They are acidic soils with  
145 pH values of 4-4.5 and have a poorly saturated cation exchange complex. More  
146 information on the experimental site is available elsewhere (Moukoui et al., 2006;  
147 Andrianarisoa et al., 2010). The incubation took place in a stand planted with 30  
148 year-old European beech trees (*Fagus sylvatica* L). Unlabelled senescent leaves  
149 were also incubated as reference material. They were harvested in October 2004, in  
150 the stand where the incubation took place. The leaves were collected at the same  
151 height (~1.5 m) from the branches of several different trees in order to obtain mean  
152 composition and to minimize biases linked to intrapopulational variability.

153

### 154 2.2.2. Selection of senescent leaves

155 After harvesting, leaves were rinsed with distilled water to remove extraneous  
156 particles and dried at 45°C until they attained a constant weight. Leaves were then  
157 examined under a dissecting microscope to monitor morphological changes. One  
158 milligram was removed from each leaf for elemental and isotope analyses (see  
159 section 2.3.1) to determine the main biochemical characteristics of the leaves at the  
160 initial stage of degradation. Each batch was composed of 10-15 leaves and  
161 corresponded to approximately one gram of organic matter. Batches were assembled  
162 using leaves from as many different trees/plants as possible to obtain equivalent  
163 batches.  $^{13}\text{C}$ -enriched leaves were selected to constitute batches having isotope

164 variabilities similar to those of natural leaves (i.e. a standard deviation  $\leq 0.8$  ‰). The  
165 mean isotope composition of the batches of labelled leaves varied within a rather  
166 large range (i.e. +16.3 ‰ to +36.8 ‰) that precluded direct comparison between  
167 batches. Moreover, labelled and unlabelled leaves were grown in different conditions  
168 and exhibited slightly different biochemical characteristics. Therefore, to avoid biases  
169 linked to differences between the  $^{13}\text{C}$ -enriched leaf batches and the wild leaf batches  
170 at the initial stage, the following discussion will be based primarily on the comparison  
171 of degradation trends (i.e.  $\Delta^{13}\text{C}_{\text{degradation}} = \delta^{13}\text{C}_{\text{degraded}} - \delta^{13}\text{C}_{\text{initial}}$ ).

172

### 173 2.2.3. Litterbag incubation

174 Preliminary investigations showed that in contrast to aluminium nets, the nylon  
175 and polypropylene nets generally used for litterbags may release substantial  
176 amounts of contaminants that could be potentially extracted with the leaf lipids.  
177 Accordingly, the litterbags (9.5 × 14.5 cm) were made of aluminium wire. The mesh  
178 size was 1.4 × 1.8 mm and was small enough to contain small leaf litter debris yet  
179 large enough to permit aerobic microbial activity and free entry of small soil animals.  
180 Indeed, soil fauna at the experimental site was dominated in biomass by  
181 enchytraeids, and contained to a lesser extent, mites and springtails, as it is  
182 generally the case in moders (Swift et al., 1979; Ponge et al., 1997). Before being  
183 enclosed in litterbags, the leaves were weighed and then re-moistened with distilled  
184 water to recreate the natural moisture conditions of leaf fall in autumn. The litterbags  
185 were placed in the field at the end of autumn 2004, on the surface of the existing  
186 forest litter layer, akin to leaves falling naturally in autumn. Samples were retrieved  
187 from the field at 5, 10, 15, 20, 30, 40, 52, 79, 129 and 207 weeks (four years). At  
188 each sampling date, two bags of natural leaves and two bags of labelled leaves were  
189 taken.

190

### 191 2.3. Analyses

192

193 The recovered material was first rinsed with distilled water to remove obvious  
194 extraneous particles such as mosses, roots or arthropods. Leaves were then  
195 scanned, dried to constant weight at 45°C, weighed and examined under a dissecting  
196 microscope. Samples were stored dark in aluminium foil at 5°C until analysis. All the

197 leaves of each litterbag were then combined and ground fine enough to pass through  
198 a 500  $\mu\text{m}$  mesh. An aliquot of the so-obtained powder ( $\sim 1$  mg) was taken for bulk  
199 elemental and isotope characterization (see 3.2.1.) and the rest was submitted to the  
200 extraction procedure.

201

### 202 2.3.1. Isotope and elemental analyses of bulk leaves

203 Stable isotope ratios were measured using an automated unit that combines  
204 an elemental analyser with an isotope ratio mass spectrometer. The samples were  
205 combusted in a Carlo-Erba CHN elemental analyser connected to a VG-SIRA 10,  
206 isotope ratio mass spectrometer. After flash combustion with copper oxide at  $1000^\circ\text{C}$ ,  
207  $\text{CO}_2$  was cryogenically distilled, purified and introduced on-line into the mass  
208 spectrometer to determine carbon isotope ratios (Girardin and Mariotti, 1991). The  
209  $\delta^{13}\text{C}$  values were measured with a precision of  $0.1\text{‰}$  ( $1\sigma$ ).

210

### 211 2.3.2. Lipid extraction and alkane fractionation

212 Leaf powder was ultrasonically extracted for 20 min with 30 ml  
213 dichloromethane/methanol (2/1; v/v). The mixture was centrifuged (10 min at 4,000  
214 rpm) and the extracted lipids were recovered in the supernatant. The extraction  
215 procedure was repeated six times with the centrifugation residue (pellet). The  
216 combined extracts were concentrated by rotary evaporation and then dried under  
217 nitrogen gas. The extract yield was determined by weighing the dried combined  
218 extracts. The total extract was then fractionated on an alumina column (1 g alumina  
219 per 10 mg dry extract, Sigma-Aldrich 507C  $\sim 150$  mesh) deactivated to Brockmann  
220 grade IV by adding 0.1% by weight of distilled water. Alkanes were recovered in the  
221 apolar fraction after elution with heptane (4 ml for 1 g of alumina). A known amount of  
222 an internal standard (i.e. tetratriacontane) was introduced in the fraction for  
223 quantification purposes.

224

### 225 2.3.3. Alkane-specific isotope analyses

226 Alkanes were identified by gas chromatography using a an Agilent 6890N gas  
227 chromatograph (GC) equipped with a split/splitless injector and fitted with a fused  
228 silica capillary column, coated with VF5-MS (50 m  $\times$  0.32 mm i.d., 0.12  $\mu\text{m}$  film  
229 thickness). For GC the temperature was increased from  $80^\circ\text{C}$  to  $100^\circ\text{C}$  at  $10^\circ\text{C}/\text{min}$ ,



230 then increased to 325°C at 4°C/min, then held at 325°C for 30 min. Helium was used  
231 as carrier gas at a constant flow of 2 ml/min. The splitless injector and flame  
232 ionization detector temperatures were held at 350°C. Compound-specific  $\delta^{13}\text{C}$  values  
233 of the dominant alkanes were determined with a Micromass IsoChrom III coupling,  
234 comprised by an IsoChrom isotope ratio mass spectrometer coupled to a GC via a  
235 Micromass combustion interface (CuO combustion furnace at 850°C and cryogenic  
236 trap at -100°C).  $\delta^{13}\text{C}$  values were measured with a precision ( $1\sigma$ ) of 0.5‰. Each  
237 sample was run in triplicate. To enable comparison between leaves that exhibited a  
238 rather large range of isotope variability, the isotope compositions of specific alkanes  
239 were expressed relative to the bulk isotope composition of undegraded leaves  
240 according to:  $\Gamma^{13}\text{C}_{\text{alkane}} = \delta^{13}\text{C}_{\text{alkane}} - \delta^{13}\text{C}_{\text{undegraded leaves}}$ .

241

242

### 243 **3. Results and discussion**

244

#### 245 *3.1. Bulk degradation patterns*

246

247         Microscopic examination of the leaves revealed traces of epiphytic microflora  
248 beginning at the initial/senescent stage. Indeed, fungal fructifications (Fig. 1a) and  
249 mycelia (Fig. 1b) were detected on several leaves. During the experiment, the leaves  
250 exhibited progressive degradation patterns. The first evidence of grazing by soil  
251 fauna, in which the lower cuticle was missing between the tertiary/quaternary veins,  
252 was detected after five weeks of decomposition in the field. The lower cuticle of  
253 leaves degrades more easily than the upper cuticle and the veins are much thicker  
254 and decay more slowly. These grazing patterns are typical of litter-consuming fauna,  
255 notably the enchytraeids that are very abundant in moders (Ponge, 1999). These  
256 grazing marks became frequently observed after 10 weeks of decomposition (Fig.  
257 1c). After one year, the upper cuticle of leaves showed evidence of grazing, leading  
258 to complete holes between veins (Figs. 1d and 1e) and to a progressive  
259 skeletonization of the leaves (Fig. 1f). After decomposing for four years, the only  
260 original material remaining consisted of bundles of skeletonized leaf fragments (Fig.  
261 1f). Exogenous material also accumulated on the leaves during the experiment.  
262 Fungi progressively invaded the leaf surfaces (Fig. 1d); on leaves exposed to light,

263 micro-algae colonized the gaps left by grazing fauna (Fig. 1d); faecal material was  
264 deposited by soil fauna (Fig. 1e); and conifer pollen grains probably originating from  
265 adjacent stands were also deposited (Fig. 1e and 1f). Labelled and unlabelled leaves  
266 exhibited similar degradation patterns upon microscopic examination.

267 After four years of decomposition in the field, the litter leaves had lost 50-90%  
268 of their initial dry weight (Fig. 2a). A rather large weight loss was observed during the  
269 first several days followed by slower decomposition rates afterwards. This  
270 decomposition trend is in agreement with other studies that reported the  
271 decomposition patterns of beech litters under similar conditions (Cortez, 1998; Ono  
272 *et al.*, 2009). <sup>13</sup>C-labelled leaves appeared to degrade slightly more quickly than  
273 natural leaves during the first three years (Fig. 2a). Nevertheless, both leaf types  
274 eventually lost equivalent weights after four years (Fig. 2a). The difference in initial  
275 decomposition rates of the natural and the labelled leaves probably reflected  
276 differences in initial leaf quality. Among other factors, the C/N ratio was lower in  
277 labelled leaves than in natural leaves at the initial stage (with means of 19.6 and 26.9  
278 for labelled and unlabelled leaves, respectively). This lower C/N ratio probably  
279 contributed significantly to the difference in decomposition rate, and has been  
280 suggested previously (Melin, 1930; Witkamp, 1966; Taylor *et al.*, 1989). Litter quality  
281 differences may be attributed to growing conditions that were probably more  
282 favourable in the labelling chamber than in the field. To avoid potential bias linked to  
283 this difference in litter quality, we based our discussion on degradation trends  
284 calculated relative to the initial/undegraded stage of each batch, rather than on direct  
285 comparisons between the two crude datasets. Regardless of the dataset, no specific  
286 trends were evident with respect to the carbon content or the C/N ratio, both of which  
287 remained relatively constant during the experiment (~450 mg/g and ~20,  
288 respectively; data not shown). These values agree with those previously reported for  
289 beech leaves and litter found at the experimental site (Zeller *et al.*, 2007).

290 Lipids of senescent leaves (i.e. at the initial stage of degradation) accounted  
291 for ~11 wt.% and ~16 wt.% of the dry material for unlabelled and <sup>13</sup>C-labelled leaves,  
292 respectively. Here again, the higher lipid content of the labelled leaves compared to  
293 that of the natural leaves was probably due to differences in growing conditions.  
294 Nevertheless, both leaf types exhibited similar degradation patterns for lipids (Fig.  
295 2b). Lipid content decreased sharply during the first month of the experiment,  
296 reaching ~6 wt.% of dry material. Leaf lipid content then progressively decreased to

297 become >1 wt.% after four years of degradation (Fig. 2b). Final lipid loss was greater  
298 than 99 wt.% of the initial lipid content, which was much higher than the bulk weight  
299 loss (i.e. ~80 wt.% of initial leaves). Accordingly, lipids were degraded to a greater  
300 extent than were the non-lipidic constituents of the leaves. The chemical composition  
301 of lipids from senescent beech leaves has been reported previously (Nguyen Tu et  
302 al., 2007): most *n*-alkanes consisted of between 16 and 29 carbon atoms and were  
303 markedly dominated by the C<sub>27</sub> homologue. The initial alkane content present in  
304 senescent leaves decreased substantially during the four year decomposition, as  
305 illustrated by the amount of the C<sub>27</sub> homologue (Fig. 2c).

306

### 307 3.2. Isotope degradation patterns

308

309 The bulk isotope composition of unlabelled leaves remained nearly constant  
310 during decomposition, whereas that of the <sup>13</sup>C-labelled leaves exhibited a significant  
311 decrease over time (i.e. <sup>13</sup>C-depletion, Fig. 3a). This <sup>13</sup>C-depletion reached 12‰ after  
312 the four-year decomposition period. In agreement with previous reports (Collister et  
313 al., 1994; Chikaraishi et al., 2004), specific alkanes appeared to be <sup>13</sup>C-depleted  
314 compared to the bulk leaves. The <sup>13</sup>C-content of specific alkanes exhibited  
315 degradation patterns similar to that of bulk leaves. For example, the <sup>13</sup>C-content of  
316 the C<sub>27</sub> homologue (Fig. 3b) remained nearly constant in the unlabelled leaves but  
317 exhibited a decrease ~20‰ in the <sup>13</sup>C-enriched leaves. The C<sub>25</sub> and C<sub>29</sub> homologues  
318 exhibited degradation patterns similar to that of C<sub>27</sub> (data not shown). At the bulk leaf  
319 scale, as well as at the molecular scale, previous authors observed either increasing  
320 δ<sup>13</sup>C values or unchanging isotope content during organic matter degradation  
321 (Balesdent et al., 1993; Connin et al., 2001). These studies dealt with unlabelled  
322 material and reported <sup>13</sup>C-enrichments of up to 6‰. <sup>13</sup>C-depletion in the context of  
323 unlabelled organic matter decomposition was reported in only one early study of bulk  
324 *Spartina* rhizomes decaying in salt marsh sediments (Benner et al., 1987). The  
325 observed variability in the trends of <sup>13</sup>C content that has been documented in the  
326 literature is likely due to the wide range of environments employed to investigate  
327 decomposition. In a recent experiment dealing with highly <sup>13</sup>C-enriched (i.e. δ<sup>13</sup>C ≈  
328 166‰) poplar leaves, Rubino and colleagues (2010) reported a decrease of 41‰ in  
329 δ<sup>13</sup>C after one year of litter degradation.

330 Disproportionate changes in isotope content during degradation may reflect  
331 either contributions from exogenous material having different isotope compositions or  
332 an "isotope effect" (Natelhoffer and Fry, 1988; Balesdent et al., 1993). The latter  
333 phenomenon describes the selective degradation of chemical compounds having  
334  $\delta^{13}\text{C}$  values deviating from that of bulk leaves and/or kinetic discrimination during  
335 metabolism of leaf constituents by degrading microorganisms (i.e. preferential  
336 metabolism of the material that is  $^{13}\text{C}$ -depleted). Kinetic discrimination would be  
337 expected to generate similar patterns for a given species in a given environment.  
338 This explanation can thus be ruled out in our experiment since  $^{13}\text{C}$ -labelled and  
339 unlabelled leaves exhibited different isotope depletion patterns. The preferential  
340 degradation of the lipids noted in section 3.1 did not likely account for the observed  
341 isotope trends because lipids corresponded to only between 1 and 15% of the bulk  
342 leaf weight. Moreover, lipids are  $^{13}\text{C}$ -depleted compared to the bulk leaves (Park and  
343 Epstein, 1960; Monson and Hayes, 1982). Selective degradation of  $^{13}\text{C}$ -enriched  
344 molecules such as carbohydrates may partly explain the isotope content trends  
345 detected in bulk leaves. Indeed,  $^{13}\text{C}$ -labelled leaves were grown in optimal conditions  
346 which may have led to them having higher carbohydrate content than the unlabelled  
347 leaves. Since carbohydrates can be  $^{13}\text{C}$ -enriched up to 5‰ with respect to bulk  
348 leaves (Ehleringer, 1991), this may have played a role in the different behaviours of  
349 the isotope content in labelled and unlabelled leaves. Nevertheless, the depletion  
350 observed here for bulk labelled leaves reached 12‰ after four years of degradation.  
351 A complementary explanation is thus necessary to fully explain the observed isotope  
352 patterns, especially with respect to those observed for specific alkanes. Accordingly,  
353 this is most likely due to a contribution from exogenous organic matter that is of a  
354  $\delta^{13}\text{C}$  value lower than that of the labelled leaves. Such an exogenous contribution  
355 may be linked to the progressive invasion and/or accumulation of algae, faeces and  
356 fungi which were revealed upon microscopic examination. Although such soil-derived  
357 microbial biomass is generally considered to be devoid of long chain lipids such as *n*-  
358 alkanes, microbes such as fungi or algae may contain long-chain lipids (Jones and  
359 Young, 1970; Rezanka and Sokolov, 1993; Volkman et al., 1998).

360 The isotope composition of the exogenous organic matter that may have  
361 contributed to the decaying leaves is likely similar to or slightly enriched in  $^{13}\text{C}$  with  
362 respect to the original unlabelled material, whether at the bulk leaf level or the  
363 molecular level. Indeed, soil microbial biomass is generally considered to be  $^{13}\text{C}$ -

364 enriched by 1-3‰ compared to soil organic matter (Werth and Kuzyakov, 2010; and  
365 reference therein). Fungi can be either slightly <sup>13</sup>C-depleted or <sup>13</sup>C-enriched relative  
366 to the <sup>13</sup>C content of the substrate, depending on the fungus taxon and on the  
367 substrate (Gleixner et al., 1993; Hobbie et al., 2001; Abraham and Hesse, 2003;  
368 Ruess et al., 2005; Cowie et al., 2009). The sporophores of the saprophytic fungi  
369 from our experimental site were shown to be <sup>13</sup>C-enriched by ~4‰ with respect to  
370 their substrate (Zeller et al., 2007). Specific lipids from heterotrophic microorganisms  
371 have been shown to be either <sup>13</sup>C-enriched or <sup>13</sup>C-depleted by a few per mill, with  
372 respect to their total biomass, according to the taxon and to the environment (Teece  
373 et al., 1999; Abraham and Hesse, 2003; Cowie et al., 2009). The carbon isotope  
374 composition of faeces relative to diet is poorly known for soil fauna and seems to  
375 vary among taxa for small metazoans (DeNiro and Epstein, 1978; Tamelander et al.,  
376 2006). Specific compounds of faeces from soil fauna may reflect that of their diet as  
377 shown for copepod sterols (Grice et al., 1998). Therefore, the exogenous contribution  
378 to the original plant material probably had an isotope composition similar to or slightly  
379 higher than that of the unlabelled leaves. This implies that fungi, other microbes and  
380 the animals that produced the faeces in the litter likely derived mostly their diet from  
381 unlabelled organic matter that was the main substrate in the experimental site.

382 By comparing degradation patterns in <sup>13</sup>C-labelled and unlabelled leaves, this  
383 study provides evidence for microbial contributions to decaying litter at the level of  
384 bulk organic material and at the level of specific alkanes. This exogenous  
385 contribution was probably substantive but relatively small when compared to the bulk  
386 mass of the leaves in our samples. In this way, no isotope trend would be expected  
387 for unlabelled leaves while <sup>13</sup>C-enriched leaves would be expected to exhibit  
388 decreasing  $\delta^{13}\text{C}$  values throughout the decay period. While contribution from  
389 microbial organic matter to bulk plant organic matter has been well established  
390 previously, this has not been reported for long-chain alkanes. These molecules have  
391 been generally considered to be typical of plants, although recent studies suggested  
392 that microbial alkanes may contribute to degrading plant alkanes in soils and  
393 sediments (Buggle et al., 2010). The present study thus provides direct isotope  
394 arguments in favour of microbial contribution rather than isotope discrimination  
395 effects to explain the <sup>13</sup>C-enrichment trends previously reported for plant alkanes in  
396 soils (Nguyen Tu et al., 2004; Chikaraishi and Naraoka, 2006). Although microbial  
397 contribution appeared to be relatively limited for unlabelled/natural litter for this four-

398 year-incubation, results from other studies suggest that it may lead to a  $^{13}\text{C}$ -  
399 enrichment of up to 4‰ (Chikaraishi and Naraoka, 2006). This result was actually  
400 based on lipids extracted from bulk soils sampled from the horizon underlying the  
401 litter layer (i.e. 2-10 cm in depth). Therefore, it may be suggested that the high  $^{13}\text{C}$ -  
402 enrichment detected by Chikaraishi and Naraoka (2006) corresponded to the  
403 accumulation over time of an increase in  $^{13}\text{C}$ -content that was negligible at first but  
404 that led to a measurable effect in the long-term. Chikaraishi and Naraoka (2006)  
405 found that long-chain *n*-alkanols and *n*-alkanoic acids can also undergo significant  
406  $^{13}\text{C}$ -enrichment during leaf degradation in soils. We may therefore generalize our  
407 findings to other long-chain fatty lipids, which may also be contaminated by microbial  
408 lipids. Thus, circumspection is called for when considering long-chain fatty lipids as  
409 unaltered record of organic carbon isotope composition.

410

411

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413

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595

596 **Figure captions**

597

598 **Figure 1.**

599 Typical beech leaf decomposition patterns observed under dissecting microscope.

600 Epiphytic fungi on senescent leaves: fructifications (**a.**) and mycelia (**b.**). Marks of

601 grazing by soil fauna after 1.5 years of decomposition (**c.**). Decomposition patterns

602 after 2.5 years of decay: skeletonization of leaves (**d.** arrow H and **f.**), increasing

603 invasion by fungi (**d.**) and algae (**d.** arrow A), faecal material (**e.** arrow F) and conifer

604 pollen grains (**e.** arrows P and **f.** arrows)

605

606 **Figure 2.**

607 Changes in geochemical parameters measured for bulk beech leaves.

608 Solid symbols: unlabelled leaves, open symbols:  $^{13}\text{C}$ -labelled leaves. **a.** Bulk weight

609 (percentage of initial weight). **b.** Lipid content (percentage of initial leaf weight). **c.**

610 Heptacosane content (with respect to initial leaf weight).

611

612

613 **Figure 3.**

614 Changes in isotope composition.

615 Solid symbols: unlabelled leaves; open symbols:  $^{13}\text{C}$ -labelled leaves. **a.**  $^{13}\text{C}$ -content

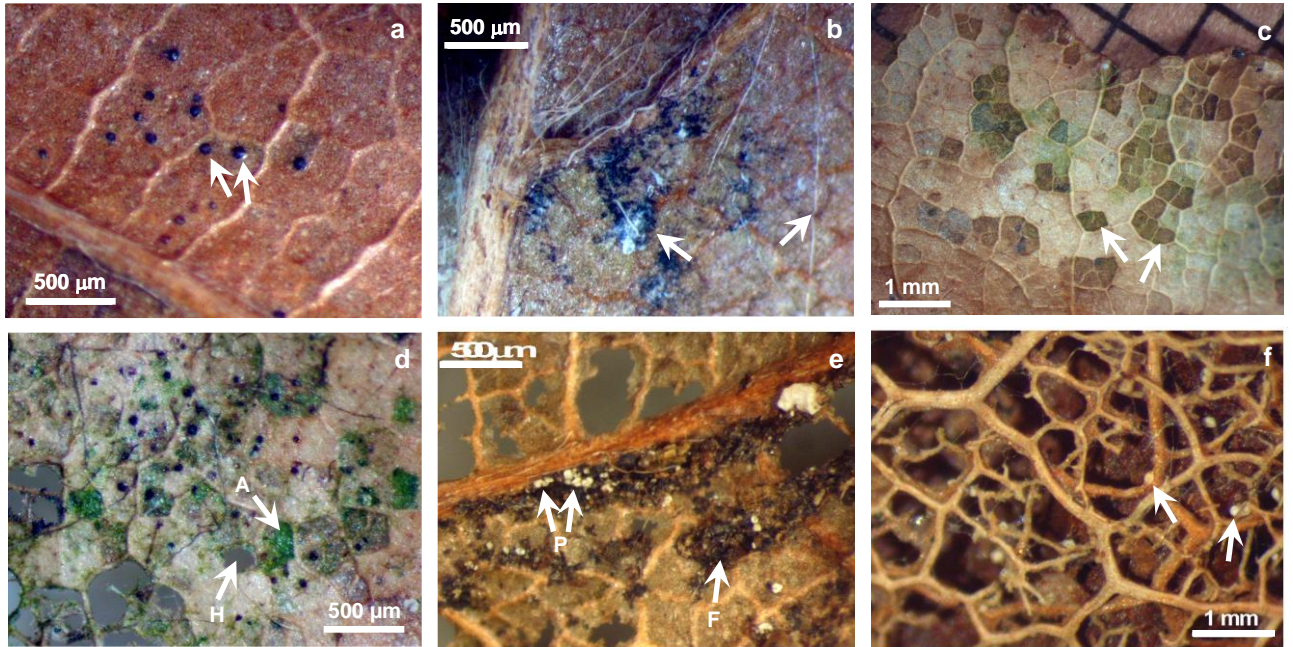
616 of bulk leaves ( $\Delta^{13}\text{C} = \delta^{13}\text{C}_{\text{degraded}} - \delta^{13}\text{C}_{\text{initial}}$ ). **b.** Specific  $^{13}\text{C}$ -content of heptacosane

617 ( $\Gamma^{13}\text{C} = \delta^{13}\text{C}_{\text{heptacosane}} - \delta^{13}\text{C}_{\text{bulk undegraded leaves}}$ ).

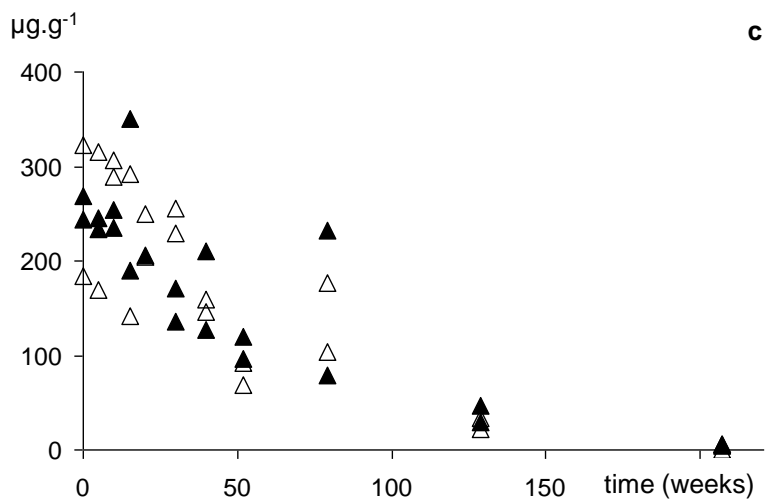
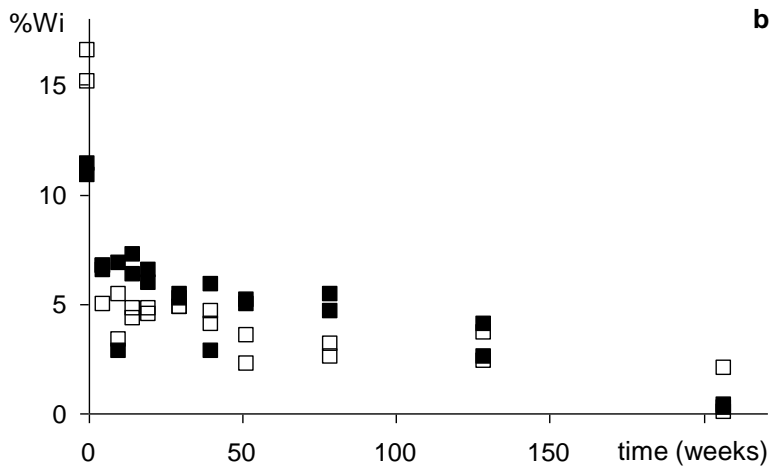
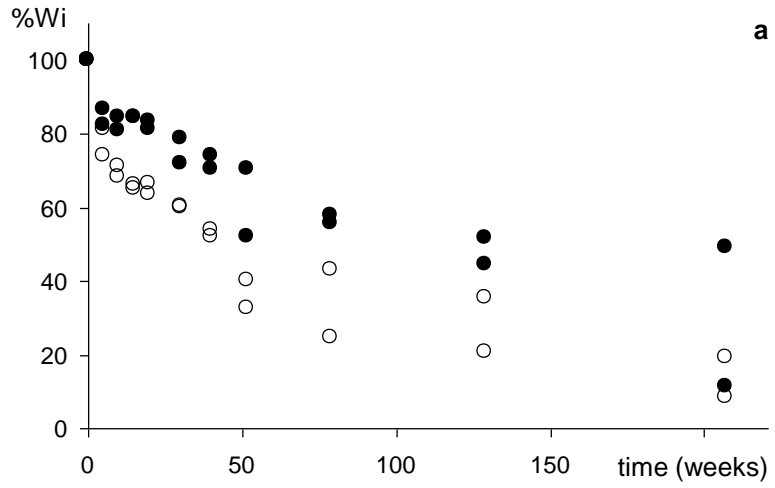
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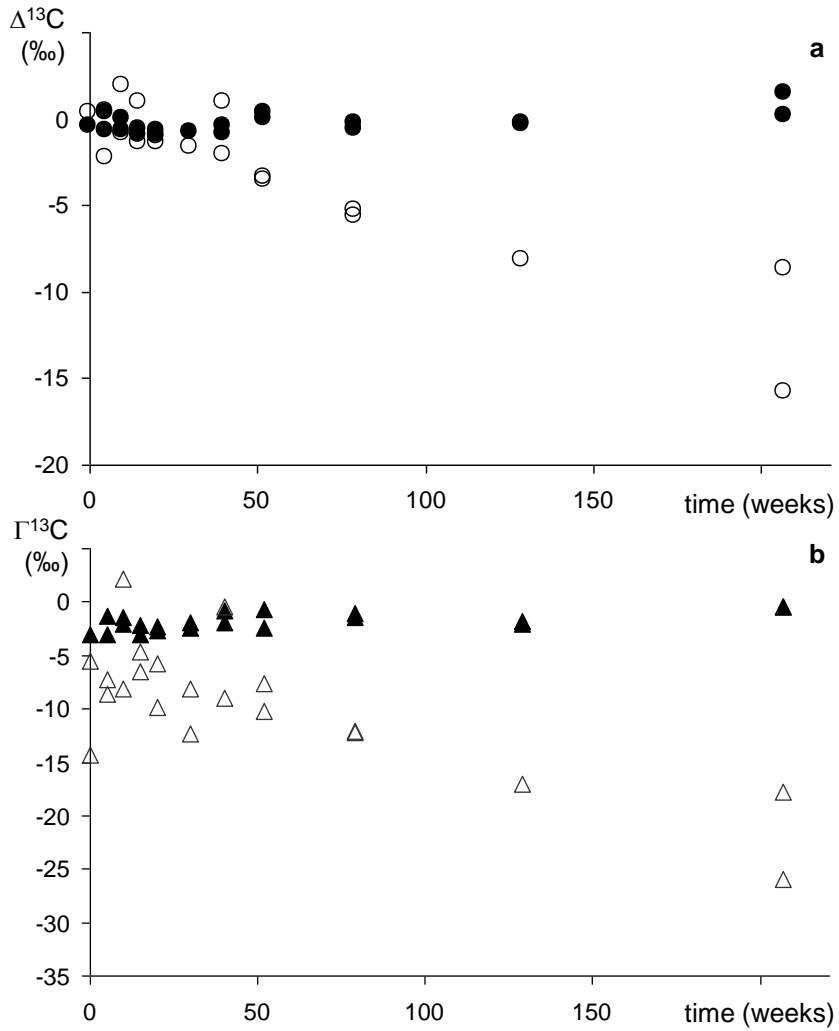
Nguyen Tu et al. Figure 1



Nguyen Tu et al. Figure 2



Nguyen Tu et al. Figure 3



Nguyen Tu et al. Graphical abstract

