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1 **Early degradation of plant alkanes in soils:**
2 **A litterbag experiment using ¹³C-labelled leaves**

3
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19

20 **Highlights:**

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

27

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}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}C -enriched leaves exhibited a
35 marked decrease in ^{13}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}C -content of these compounds was unaffected over
69 time or it exhibited a trend towards ^{13}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}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}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}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}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}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}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}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}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 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-Chenue"
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 (Moukoumi 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}C -enriched leaves were selected to constitute batches having isotope

164 variabilities similar to those of natural leaves (i.e. a standard deviation ≤ 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}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 μm mesh. An aliquot of the so-obtained powder (~ 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°C ,
207 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‰ (1σ).

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 ~ 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 μm film
229 thickness). For GC the temperature was increased from 80°C to 100°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 (1σ) 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). ¹³C-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 ¹³C-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 C₂₇ 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 C₂₇ 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 ¹³C-labelled leaves exhibited a significant
311 decrease over time (i.e. ¹³C-depletion, Fig. 3a). This ¹³C-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 ¹³C-depleted
314 compared to the bulk leaves. The ¹³C-content of specific alkanes exhibited
315 degradation patterns similar to that of bulk leaves. For example, the ¹³C-content of
316 the C₂₇ homologue (Fig. 3b) remained nearly constant in the unlabelled leaves but
317 exhibited a decrease ~20‰ in the ¹³C-enriched leaves. The C₂₅ and C₂₉ homologues
318 exhibited degradation patterns similar to that of C₂₇ (data not shown). At the bulk leaf
319 scale, as well as at the molecular scale, previous authors observed either increasing
320 δ¹³C 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 ¹³C-enrichments of up to 6‰. ¹³C-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 ¹³C 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 ¹³C-enriched (i.e. δ¹³C ≈
328 166‰) poplar leaves, Rubino and colleagues (2010) reported a decrease of 41‰ in
329 δ¹³C 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}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}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}C -depleted compared to the bulk leaves (Park and
343 Epstein, 1960; Monson and Hayes, 1982). Selective degradation of ^{13}C -enriched
344 molecules such as carbohydrates may partly explain the isotope content trends
345 detected in bulk leaves. Indeed, ^{13}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}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}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}C -

364 enriched by 1-3‰ compared to soil organic matter (Werth and Kuzyakov, 2010; and
365 reference therein). Fungi can be either slightly ¹³C-depleted or ¹³C-enriched relative
366 to the ¹³C 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 ¹³C-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 ¹³C-enriched or ¹³C-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 ¹³C-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 ¹³C-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 ¹³C-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}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}C -
402 enrichment detected by Chikaraishi and Naraoka (2006) corresponded to the
403 accumulation over time of an increase in ^{13}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}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}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}C -labelled leaves. **a.** ^{13}C -content

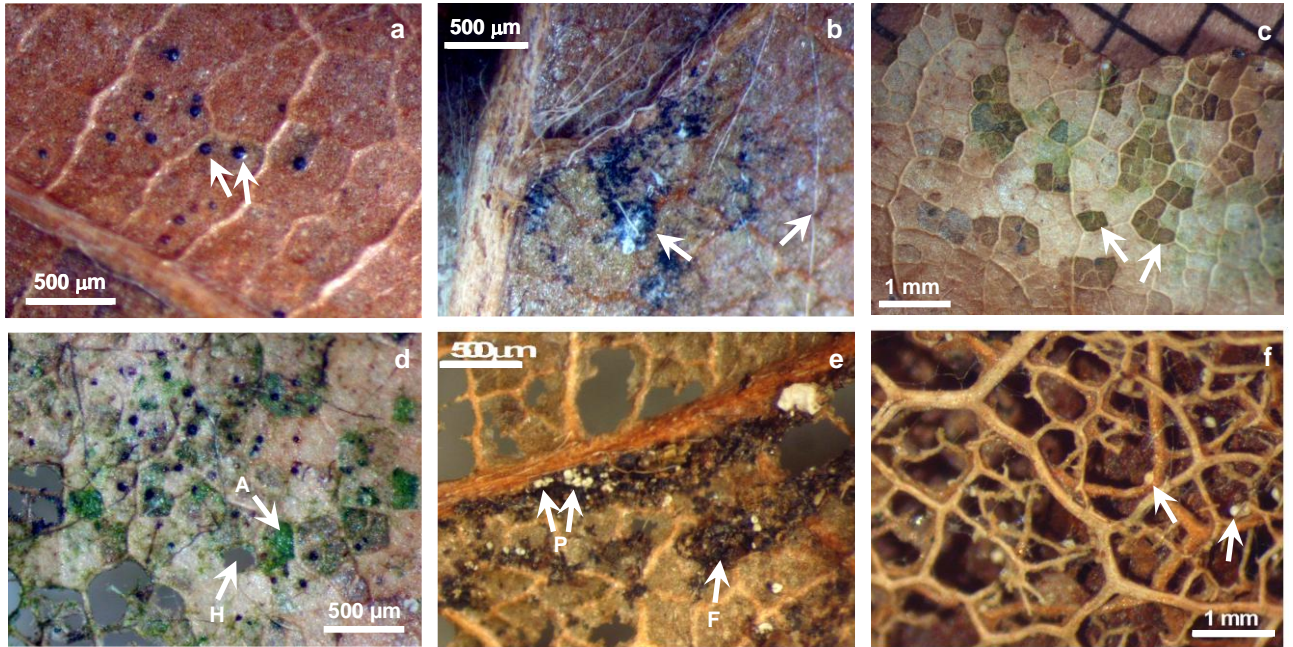
616 of bulk leaves ($\Delta^{13}\text{C} = \delta^{13}\text{C}_{\text{degraded}} - \delta^{13}\text{C}_{\text{initial}}$). **b.** Specific ^{13}C -content of heptacosane

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

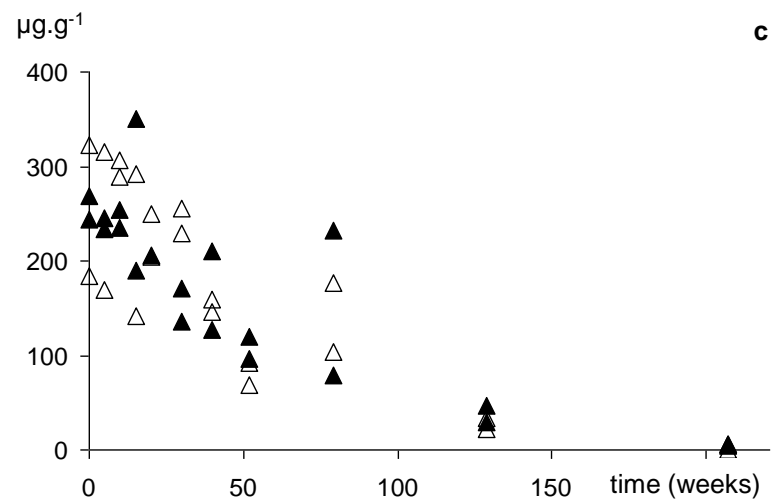
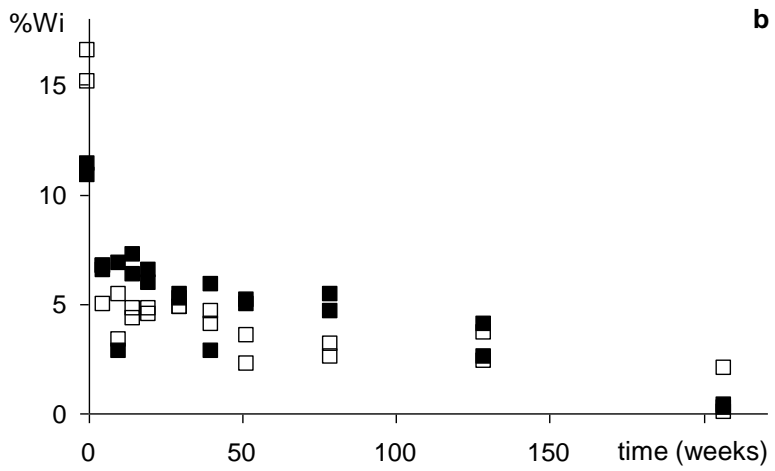
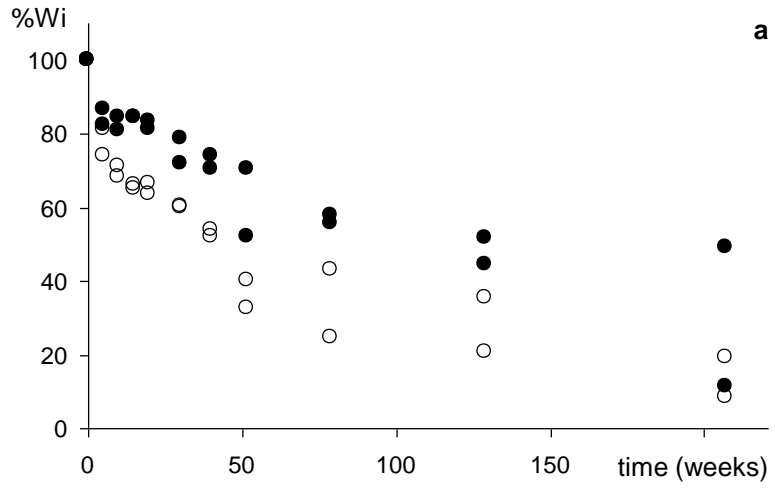
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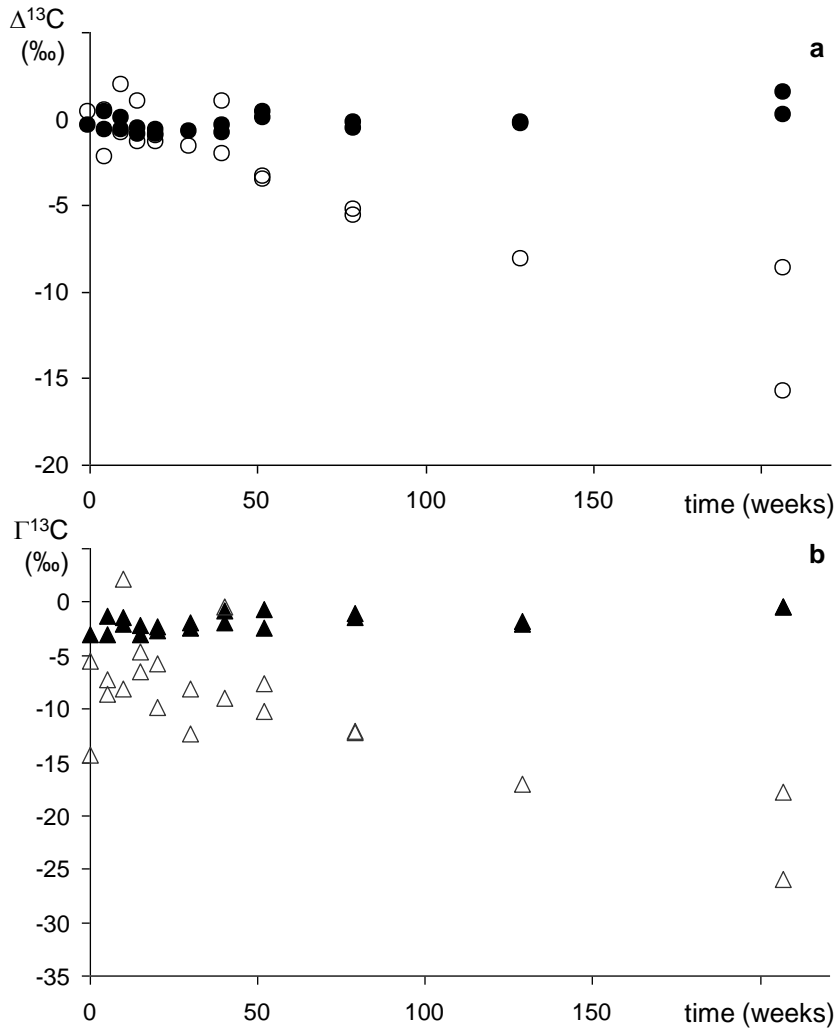
Nguyen Tu et al. Figure 1



Nguyen Tu et al. Figure 2



Nguyen Tu et al. Figure 3



Nguyen Tu et al. Graphical abstract

