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Early degradation of plant alkanes in soils:

A litterbag experiment using ^{13}C -labelled leaves

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Highlights:

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

Abstract

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

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

1. Introduction

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

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

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

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

2. Material and methods

2.1. ^{13}C -labelling of beech leaves

Beech seeds were germinated at the end of May 2004. Young seedlings were transferred at the cotyledon stage to a controlled chamber at the beginning of June 2004. In order to minimize isotope effects linked to soil respiration, plants were grown on vermiculite and fed three times per week with a previously described nutritional solution (Coïc and Lesaint, 1983). The pCO_2 and $\delta^{13}\text{C}_{\text{atm}}$ were maintained at 380 ppmv and +40‰, respectively. Climate conditions in the chamber were held constant during the five months of labelling: 70% relative humidity and 20°C day temperature. Four Osram HQI/D Daylight lamps (400 W/D) provided a light intensity of approximately $350 \mu\text{mol}/\text{m}^2\cdot\text{s}$ of photosynthetically active radiation during a 15 hr period per day (i.e. 7h-20h). The chamber is described in detail elsewhere (Girardin et al., 2009). Three weeks before the end of the experiment, the conditions were modified in order to induce leaf senescence. The temperature was reduced to 18°C,

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

2.2. Degradation experiment

2.2.1. Experimental site

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

2.2.2. Selection of senescent leaves

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

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

2.2.3. Litterbag incubation

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

2.3. Analyses

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

leaves of each litterbag were then combined and ground fine enough to pass through a 500 μm mesh. An aliquot of the so-obtained powder (~ 1 mg) was taken for bulk elemental and isotope characterization (see 3.2.1.) and the rest was submitted to the extraction procedure.

2.3.1. Isotope and elemental analyses of bulk leaves

Stable isotope ratios were measured using an automated unit that combines an elemental analyser with an isotope ratio mass spectrometer. The samples were combusted in a Carlo-Erba CHN elemental analyser connected to a VG-SIRA 10, isotope ratio mass spectrometer. After flash combustion with copper oxide at 1000°C , CO_2 was cryogenically distilled, purified and introduced on-line into the mass spectrometer to determine carbon isotope ratios (Girardin and Mariotti, 1991). The $\delta^{13}\text{C}$ values were measured with a precision of 0.1‰ (1σ).

2.3.2. Lipid extraction and alkane fractionation

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

2.3.3. Alkane-specific isotope analyses

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

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

3. Results and discussion

3.1. Bulk degradation patterns

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

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

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

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

become >1 wt.% after four years of degradation (Fig. 2b). Final lipid loss was greater than 99 wt.% of the initial lipid content, which was much higher than the bulk weight loss (i.e. ~80 wt.% of initial leaves). Accordingly, lipids were degraded to a greater extent than were the non-lipidic constituents of the leaves. The chemical composition of lipids from senescent beech leaves has been reported previously (Nguyen Tu et al., 2007): most *n*-alkanes consisted of between 16 and 29 carbon atoms and were markedly dominated by the C₂₇ homologue. The initial alkane content present in senescent leaves decreased substantially during the four year decomposition, as illustrated by the amount of the C₂₇ homologue (Fig. 2c).

3.2. Isotope degradation patterns

The bulk isotope composition of unlabelled leaves remained nearly constant during decomposition, whereas that of the ¹³C-labelled leaves exhibited a significant decrease over time (i.e. ¹³C-depletion, Fig. 3a). This ¹³C-depletion reached 12‰ after the four-year decomposition period. In agreement with previous reports (Collister et al., 1994; Chikaraishi et al., 2004), specific alkanes appeared to be ¹³C-depleted compared to the bulk leaves. The ¹³C-content of specific alkanes exhibited degradation patterns similar to that of bulk leaves. For example, the ¹³C-content of the C₂₇ homologue (Fig. 3b) remained nearly constant in the unlabelled leaves but exhibited a decrease ~20‰ in the ¹³C-enriched leaves. The C₂₅ and C₂₉ homologues exhibited degradation patterns similar to that of C₂₇ (data not shown). At the bulk leaf scale, as well as at the molecular scale, previous authors observed either increasing δ¹³C values or unchanging isotope content during organic matter degradation (Balesdent et al., 1993; Connin et al., 2001). These studies dealt with unlabelled material and reported ¹³C-enrichments of up to 6‰. ¹³C-depletion in the context of unlabelled organic matter decomposition was reported in only one early study of bulk *Spartina* rhizomes decaying in salt marsh sediments (Benner et al., 1987). The observed variability in the trends of ¹³C content that has been documented in the literature is likely due to the wide range of environments employed to investigate decomposition. In a recent experiment dealing with highly ¹³C-enriched (i.e. δ¹³C ≈ 166‰) poplar leaves, Rubino and colleagues (2010) reported a decrease of 41‰ in δ¹³C after one year of litter degradation.

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

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

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

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

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

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595

Figure captions

Figure 1.

Typical beech leaf decomposition patterns observed under dissecting microscope. Epiphytic fungi on senescent leaves: fructifications (**a.**) and mycelia (**b.**). Marks of grazing by soil fauna after 1.5 years of decomposition (**c.**). Decomposition patterns after 2.5 years of decay: skeletonization of leaves (**d.** arrow H and **f.**), increasing invasion by fungi (**d.**) and algae (**d.** arrow A), faecal material (**e.** arrow F) and conifer pollen grains (**e.** arrows P and **f.** arrows)

Figure 2.

Changes in geochemical parameters measured for bulk beech leaves. Solid symbols: unlabelled leaves, open symbols: ^{13}C -labelled leaves. **a.** Bulk weight (percentage of initial weight). **b.** Lipid content (percentage of initial leaf weight). **c.** Heptacosane content (with respect to initial leaf weight).

Figure 3.

Changes in isotope composition. Solid symbols: unlabelled leaves; open symbols: ^{13}C -labelled leaves. **a.** ^{13}C -content 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 ($\Gamma^{13}\text{C} = \delta^{13}\text{C}_{\text{heptacosane}} - \delta^{13}\text{C}_{\text{bulk undegraded leaves}}$).

Nguyen Tu et al. Figure 1

Nguyen Tu et al. Figure 2

Nguyen Tu et al. Figure 3