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Effect of leaf litter degradation and seasonality on D/H isotope ratios of *n*-alkane biomarkers

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Abstract

During the last decade, compound-specific hydrogen isotope analysis of plant leaf-wax and sedimentary *n*-alkyl lipids has become a promising tool for paleohydrological reconstructions. However, with the exception of several previous studies, there is a lack of knowledge regarding possible effects of early diagenesis on the δD values of *n*-alkanes. We therefore investigated the *n*-alkane patterns and δD values of long-chain *n*-alkanes from three different C3 higher plant species (*Acer pseudoplatanus* L., *Fagus sylvatica* L. and *Sorbus aucuparia* L.) that have been degraded in a field leaf litterbag experiment for 27 months.

We found that after an initial increase of long-chain *n*-alkane masses (up to ~50%), decomposition took place with mean turnover times of 11.7 months. Intermittently, the masses of mid-chain *n*-alkanes increased significantly during periods of highest total mass losses. Furthermore, initially high odd-over-even predominances (OEP) declined and long-chain *n*-alkane ratios like $n\text{-C}_{31}/\text{C}_{27}$ and $n\text{-C}_{31}/\text{C}_{29}$ started to converge to the value of 1. While bulk leaf litter became systematically D-enriched especially during summer seasons (by ~8‰ on average over 27 months), the δD values of long-chain *n*-alkanes reveal no systematic overall shifts, but seasonal variations of up to 25‰ (*Fagus*, $n\text{-C}_{27}$, average ~13‰).

Although a partly contribution by leaf-wax *n*-alkanes by throughfall cannot be excluded, these findings suggest that a microbial *n*-alkane pool sensitive to seasonal variations of soil water δD rapidly builds up. We propose a conceptual model based on an isotope mass balance calculation that accounts for the decomposition of plant-derived *n*-alkanes and the build-up of microbial *n*-alkanes. Model results are in good agreement with measured *n*-alkane δD results. Since microbial ‘contamination’ is not necessarily discernible from *n*-alkane concentration patterns alone, care may have to be taken not to over-interpret δD values of sedimentary *n*-alkanes. Furthermore, since leaf-water is generally D-enriched compared to soil and lake waters, soil and water microbial *n*-alkane pools may help explain why soil and sediment *n*-alkanes are D-depleted compared to leaves.
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1. INTRODUCTION

During the last decade compound-specific hydrogen isotope ratios (δD) of plant-derived *n*-alkanes in sediments and soils have become a popular paleoclimate proxy for

reconstructing δD values of paleoprecipitation and for determining paleoaridity (Huang et al., 2004; Sachse et al., 2004; Xie et al., 2004; Liu and Huang, 2005; Pagani et al., 2006; Mügler et al., 2008; Zech et al., 2010b). This boom is based on several factors:

- The isotopic composition of meteoric water (δD and $\delta^{18}\text{O}$) was found to depend on climate parameters such

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as temperature, continentality and precipitation amount (Craig, 1961; Dansgaard, 1964).

- Albeit with a biosynthetic fractionation factor, plant photosynthetic products have the potential to record climate signals because their δD values are related to the source water δD values (Sternberg, 1988; Sessions et al., 1999; Sauer et al., 2001; Sachse et al., 2004).
- *n*-Alkanes are considered to be relatively stable against degradation (Lichtfouse et al., 1998) and alkyl hydrogen atoms are less prone to exchange reactions in comparison with other biomarkers in geologically young, thermally immature sediments (Sessions et al., 2004; Pedentchouk et al., 2006; Dawson et al., 2007).
- *n*-Alkanes originate from specific organisms and hence have the potential to serve as biomarkers (molecular fossils). For instance, long-chain *n*-alkanes with odd-over-even predominance (OEP) originate from terrestrial plant leaf waxes (Eglinton and Hamilton, 1967; Kolattukudy, 1976), whereas short- and mid-chain *n*-alkanes in lacustrine sediments often serve as aquatic biomarkers (Ficken et al., 2000; Zech et al., 2009b; Aichner et al., 2010).
- The methodological improvements allowed the online-coupling of gas chromatographs via a pyrolysis oven to isotope ratio mass spectrometers (GC-Py-IRMS) (Burgoyne and Hayes, 1998; Hilkert et al., 1999).

For a more detailed review about hydrogen isotopes (D/H) in sedimentary organic matter the reader is referred to Schimmelmann et al. (2006).

Several recent studies have identified various potential problems with interpretation of the δD values of sedimentary *n*-alkanes. First, several authors reported large interspecies δD differences under the same climatic conditions (Liu et al., 2006; Smith and Freeman, 2006; Hou et al., 2007; Feakins and Sessions, 2010). These results provide evidence that care must be taken when interpreting the δD values in the absence of knowledge about vegetation history. Second, within one plant species, variations among different *n*-alkanes were found to be up to 50‰. Furthermore, pronounced seasonal δD leaf-wax *n*-alkane shifts occur with up to 40‰ (Pedentchouk et al., 2008; Sachse et al., 2009), which can be attributed to short turnover times and suggests that δD values of leaf litter being deposited on soils or in sediments only reflect the climatic conditions of the last weeks before leaf senescence. Third, the limited δD data from plant–soil/sediment systems (Chikaraishi and Naraoaka, 2006; Sachse et al., 2006) indicate that long-chain *n*-alkanes of soils and sediments are depleted (by up to –57‰) compared to the fresh plant-derived *n*-alkanes. This finding can only be partly explained with the above mentioned seasonality effect and suggests that soil/sediment organic matter (SOM) formation may cause isotopic alterations, which have not yet been considered when reconstruction paleoclimatic and hydrologic conditions. Hence, detailed biodegradation and reworking experiments are needed to clarify possible isotopic modifications in plant–soil systems.

In this study we aim to address this open question by presenting and discussing the *n*-alkane concentration

patterns and compound-specific δD values of different leaf litter species, which have been decomposed in a field litterbag experiment for 27 months.

2. MATERIAL AND METHODS

2.1. Litterbag experiment and samples

The site for the decomposition experiment and further details on the design of the litterbag experiment have been described in detail in separate publications (Gerstberger et al., 2004; Don and Kalbitz, 2005; Kalbitz et al., 2006). In brief, it is located in the Fichtelgebirge (Northeast Bavaria, Germany; 50°08'35"N, 11°52'10"E) and was covered by *Picea abies* for about 160 years. Elevation is 780 m a.s.l. and soil development has resulted in a sandy loam to loamy Albic Rustic Podzol (WRB, 2006). The climate in the area is characterized by 1100 mm mean annual precipitation, a mean annual temperature of around 5 °C and a persistent snow cover during the winter season. δD values of the throughfall range from about –87‰ in the winter to –18‰ in the summer.

The litterbag experiment started in June 2001. Air-dried senescent foliage litter from five different species, including the three broad-leaf species *Acer pseudoplatanus* L. (sycamore maple), *Fagus sylvatica* L. (European beech) and *Sorbus aucuparia* L. (mountain ash) were exposed in the field for 1, 3, 5, 9, 12, 16, 21 and 27 months. The litter from coniferous species *Picea abies* L. (Karst.) (Norway spruce) and *Pinus sylvestris* L. (Scots pine) was not included in this study due to significantly lower *n*-alkane concentrations, making accurate compound-specific δD measurements impossible. The leaf litter was packed in bags made from nylon mesh and deposited on the forest floor simulating leaf litter accumulation. In order to account for the spatial variability of the decomposition processes, 12 plots (replications) were established at two neighbouring sites, resulting in 24 subsamples for each plant species at each harvesting time. The litterbags were completely covered by naturally fallen leaf litter after 1.5 years. At the end of each collection, leaf litter was cleaned manually to remove fungal hyphae, roots, shoots and insects. After drying and grinding, subsamples were combined for *n*-alkane and δD analyses. Together with the fresh non-degraded leaves, the here presented sample batch comprises 27 mixed samples in total.

Kalbitz et al. (2006) found that mass loss over the 27 months ranged from 26% (*Fagus*) to 58% (*Sorbus*). Estimating the relative contribution of cellulose and lignin and correcting for mass losses, they additionally observed a total cellulose decomposition ranging from 51% (*Fagus*) to 86% (*Sorbus*), whereas the total lignin decomposition reached only up to 11% (*Sorbus*) (Table 1).

2.2. Analytical procedures

2.2.1. *n*-Alkane quantification

n-Alkanes from the leaf litter samples were prepared according to a slightly modified procedure described by Zech and Glaser (2008) in the Laboratory of the Department of Soil Physics, University of Bayreuth, Germany.

Table 1

Total mass losses of different leaf litter species (*Acer*, *Fagus* and *Sorbus*), relative depletion of cellulose and total cellulose decomposition, relative enrichment of lignin and total lignin decomposition (from Kalbitz et al. (2006)) and relative depletion of total *n*-alkanes ($\sum(n\text{-C}_{20}$ to $n\text{-C}_{35}$) and total *n*-alkane decomposition after 27 months of leaf litter degradation.

Litter/property	<i>Acer</i>		<i>Fagus</i>		<i>Sorbus</i>	
	Fresh	Decomposed	Fresh	Decomposed	Fresh	Decomposed
Total mass losses (%)	0	43.5	0	26.0	0	57.7
Cellulose ^a	16.6	11.4	18.9	12.6	24.0	7.9
Total cellulose decomposition (%)	0	61.2	0	50.7	0	86.1
Lignin ^b	11.0	25.3	20.2	27.2	16.0	33.5
Total lignin decomposition (%)	0	0 ^c	0	0.4	0	11.4
$\sum(n\text{-C}_{20}\text{--}n\text{-C}_{35})$ ($\mu\text{g/g}$)	228	85	371	85	398	125
Total <i>n</i> -alkane decomposition (%)	0	79.0	0	83.0	0	86.7

^a Van Soest procedure: acid-detergent fibre – acid-detergent lignin: % of dry weight.

^b Van Soest procedure: acid-detergent lignin: % of dry weight.

^c Indeed, a 30% accumulation was observed, which can be attributed to methodological shortcomings of the applied procedure.

Briefly, the procedure involves extraction of lipids with methanol/toluene (7/3) using an accelerated solvent extractor (ASE 200, Dionex, Germering, Germany) and purification of *n*-alkanes on silica/aluminium oxide (both 5% deactivated) columns with hexane/toluene (85/15) as eluent. Twenty micrograms of 5 α -androstane and 40 μg hexatriacontane (*n*-C₃₆) were added as internal and recovery standards, respectively. Quantification of the *n*-alkanes was performed on an HP 6890 gas chromatograph equipped with a flame ionization detector (FID). *n*-Alkane concentrations are presented in μg *n*-alkanes/g litter. Furthermore, in order to deal with the total mass losses of the litter in the litterbags, we calculated the absolute *n*-alkane masses in the litterbags and referenced them against the initial *n*-alkane masses (month 0 = 100%). Note that sample A3 (*Acer*, 5 months) revealed inexplicable anomalies in the *n*-alkane pattern as well as in the δD values and was therefore excluded from further data evaluation and illustration. Turnover times (T = mean residence times, see Table 2) for *n*-alkane decomposition were calculated based on a first-order kinetic model according to Eq. (1),

$$T = -\frac{1}{k} \quad (1)$$

where k is the decomposition rate, which is calculated according to Eq. (2).

$$k = \frac{\ln(\text{mass alkane } (t_2)) - \ln(\text{mass alkane } (t_1))}{t_2 - t_1} \quad (2)$$

Mass alkane thereby refers to the total mass of *n*-alkanes in the litterbags (referenced against the initial *n*-alkane masses (month 0 = 100%)). Data points for month 0 were excluded for the determination of the turnover times in order to account for the time lag of microbial activity.

2.2.2. Compound-specific δD analysis

δD values of *n*-C₂₇, *n*-C₂₉, and *n*-C₃₁ alkanes recovered from the leaf litter samples were determined in the Stable Isotope Laboratory at the University of East Anglia, UK using a Thermo Scientific Delta V Advantage isotope ratio mass spectrometer interfaced to a Thermo Scientific Trace

GC Isolink. Individual *n*-alkanes were separated using an Agilent J&W DB-5 column (30 m $\times$ 0.25 mm $\times$ 0.25 μm film thickness). The GC oven was programmed from 50 $^{\circ}\text{C}$ (1 min) at 20 $^{\circ}\text{C}/\text{min}$ to 150 $^{\circ}\text{C}$ (0 min), then at 6 $^{\circ}\text{C}/\text{min}$ to 300 $^{\circ}\text{C}$ (5 min). Pyrolysis conversion of organic hydrogen to H₂ was achieved at 1420 $^{\circ}\text{C}$. Hydrogen isotopic composition of *n*-alkanes is expressed relative to Vienna Standard Mean Ocean Water (VSMOW) based on an in-house reference gas adjusted daily using a squalane standard obtained from A. Schimmelmann, Indiana University, USA. In order to estimate the accuracy of our measurements, an *n*-alkane mixture (*n*-C₁₆ to *n*-C₃₀, from A. Schimmelmann) was run two times a day. The root-mean-squared (RMS) error of all these external standard analyses is $\pm 5.9\text{‰}$ for the *n*-alkanes *n*-C₂₅, *n*-C₂₇, and *n*-C₂₉ and no systematic trends over time are revealed. Standard deviations for duplicate δD -measurements of the sample *n*-alkanes are $\pm 2.4\text{‰}$ on average and are provided in parenthesis after the mean δD -values in Table 3.

Given that the δD -values of *n*-C₂₇, *n*-C₂₉, and *n*-C₃₁ revealed seasonal variations, we employed 5 two-tailed student's *t*-tests (type two-sample unequal variances) using Excel-software to estimate the confidence of these variations for each of the investigated *n*-alkanes. Matrix A comprised the means of the most prominent (least negative) δD -values of the three summer seasons; matrix B comprised the means of the most prominent (negative) δD -values of the two winter seasons.

2.2.3. Bulk δD analysis

Bulk δD values of the leaf litter samples were determined in the Laboratory of Isotope Biogeochemistry of the Bayreuth Center of Ecology and Environmental Research (University of Bayreuth, Germany). For the thermal conversion, a TC/EA oven (HEKAtech, Wegberg, Germany) was coupled via a ConFlo III Interface (Thermo Fisher Scientific, Bremen, Germany) with a Delta V Plus isotope ratio mass spectrometer (Thermo Fisher Scientific). The standard deviation of bulk δD analyses is typically less than $\pm 2\text{‰}$. All δD values are expressed in per mil (‰) relative to the Vienna Standard Mean Ocean Water

Table 2
Rates of decomposition for a first order decay, coefficients of correlation and turnover times for mid- and long-chain *n*-alkanes of three leaf litter species (*Acer*, *Fagus* and *Sorbus*) during 27 months of leaf litter degradation.

	<i>Acer</i>			<i>Fagus</i>			<i>Sorbus</i>			Mean		
	$\sum(n-C_{20}-n-C_{34})$	$n-C_{27}$	$n-C_{29}$	$\sum(n-C_{20}-n-C_{24})$	$n-C_{27}$	$n-C_{29}$	$\sum(n-C_{20}-n-C_{24})$	$n-C_{27}$	$n-C_{29}$	$n-C_{31}$	$\sum(n-C_{20}-n-C_{24})$	$\sum(n-C_{27}, n-C_{29}, n-C_{31})$
<i>k</i>	-0.030	-0.082	-0.069	-0.013	-0.073	-0.045	-0.045	-0.073	-0.099	-0.108	-0.029	-0.086
<i>R</i> ²	0.61	0.98	0.98	0.23	0.85	0.77	0.85	0.85	0.95	0.95	0.64	0.97
<i>T</i> (months)	33.1	12.2	14.5	80.0	13.8	22.1	13.8	13.8	10.1	9.3	34.1	11.7

k = decomposition rate (month⁻¹); *R*² = coefficient of correlation for a first order kinetics decay; *T* = turnover time (months).

(VSMOW) based on an in-house reference gas (H₂ from Rießner-Gase GmbH, 96215 Lichtenfels, Germany, purity 6.0), which was calibrated using standards obtained from IAEA (VSMOW2, SLAP2 and IAEA-CH-7).

3. RESULTS

3.1. *n*-Alkane concentrations, *n*-alkane masses and *n*-alkane patterns

Kalbitz et al. (2006) found that mass losses over 27 months range from 26% (*Fagus*) to 58% (*Sorbus*). Fig. 1A illustrates that after starting the field experiment in June 2001, the most prominent mass losses occurred during autumn 2001 and autumn 2002, whereas no or less significant mass losses took place in March and June 2002 and for September 2003.

The *n*-alkane concentration patterns of the three investigated leaf litter species reveal high abundances of long-chain *n*-alkanes in the range from *n*-C₂₅ to *n*-C₃₁ with a strong odd-over-even predominance (OEP, Fig. 2), which is typical for leaf-wax *n*-alkanes. *Acer* leaf litter is dominated by *n*-C₂₇ and *n*-C₂₉, *Fagus* leaf litter strongly by *n*-C₂₇ and *Sorbus* leaf litter by *n*-C₂₉ and smaller amounts of *n*-C₃₁. Although the concentrations of these long-chain *n*-alkanes vary substantially among the different leaf litter species, both Figs. 1B and 2 show that their concentrations decreased significantly after 27 months (on average from 154 to 37 µg/g litter). This indicates that *n*-alkanes were more rapidly decomposed compared to other plant-derived organic compounds such as lignin and cellulose (Table 1). Kalbitz et al. (2006) reported that leaf litter lignin had on average almost doubled its concentration in the organic matter after 27 months. In this study, mean turnover times for long-chain *n*-alkanes range from 9.3 to 14.5 months (Table 2). Being aware that *n*-alkanes are often considered to be highly recalcitrant, we acknowledge that apart from *n*-alkanes decomposition we cannot completely rule out *n*-alkanes losses by washing out of the litterbags. However, *n*-alkanes are highly hydrophobic and hence not soluble in pure water. Furthermore, in vitro experiments show that *n*-alkanes are in fact easily biodegradable within some few weeks (Pond et al., 2002).

Interestingly, except for *n*-C₂₇ of *Fagus*, the *n*-alkane concentrations (Fig. 1B) as well as the *n*-alkane masses (referenced against the initial *n*-alkane masses) (Fig. 1C) did not decrease immediately when leaf litter degradation started. Instead, in July and September 2001, *n*-alkane masses in the leaf litterbags increased by up to ~50% (*Acer* *n*-C₂₇ and *Sorbus* *n*-C₃₁). One may try explaining these findings by involving leaf litter sampling inhomogeneity. However, given the fact that each sample is a mixture of 24 subsamples from the field and based on the observation of the overall steady trends (except for *Fagus*, September 2001), our results suggest that there occurred either an *in situ* production or an additional input of long-chain *n*-alkanes from an external source during the first months.

Only in the spring 2002, after 9 months of leaf litter degradation, concentrations of all long-chain *n*-alkanes started to decrease dramatically and also *n*-alkane masses fell

Table 3
Bulk litter δD -values and compound-specific δD -values of individual *n*-alkanes (‰).

Months	<i>Acer</i>			<i>Fagus</i>		<i>Sorbus</i>		
	Bulk litter ^a	<i>n</i> -C ₂₇ ^b	<i>n</i> -C ₂₉ ^b	Bulk litter ^a	<i>n</i> -C ₂₇ ^b	Bulk litter ^a	<i>n</i> -C ₂₉ ^b	<i>n</i> -C ₃₁ ^b
June-01	−104.3	−153.5 (2.1)	−144.5 (6.4)	−97.5	−161.0 (1.4)	−95.0	−191.5 (3.5)	−161.5 (4.9)
July-01	−101.6	−145.0 (5.7)	−147.0 (0)	−93.7	−167.5 (0.7)	−102.8	−190.0 (4.2)	−170.5 (3.5)
September-01	−104.3	−161.5 (0.7)	−156.5 (6.4)	−95.0	−173.0 (2.8)	−104.0	−194.5 (2.1)	−177.5 (4.9)
November-01	−99.8	−148.5 (2.1) ^c	−162.0 (2.8) ^c	−90.8	−182.0 (1.4)	−103.9	−200.5 (2.1)	−179.5 (2.1)
March-02	−96.7	−162.0 (1.4)	−157.5 (0.7)	−91.1	−177.5 (0.7)	−103.9	−192.0 (2.8)	−170.0 (4.2)
June-02	−96.2	−153.5 (0.7)	−150.0 (1.4)	−88.5	−165.0 (0)	−99.2	−198.5 (0.7)	−178.5 (0.7)
October-02	−94.0	−159.0 (1.4)	−160.0 (0)	−89.5	−173.0 (5.7)	−92.4	−203.5 (2.1)	−183.0 (0)
March-03	−96.5	−156.0 (2.8)	−155.5 (3.5)	−88.5	−169.0 (0)	−97.3	−199.0 (2.8)	−178.5 (0.7)
September-03	−90.2	−146.0 (1.4)	−151.0 (7.1)	−90.6	−157.0 (5.7)	−92.3	−192.5 (0.7)	−182.5 (2.1)

^a Standard deviation as assessed by standards typically less than $\pm 2\%$.

^b Mean of duplicate measurements, standard deviations are given in parentheses.

^c Excluded from further data evaluation and illustration.

considerably below the initial values until October 2002. After the rate of decrease slowed down in winter 2002/2003 (Fig. 1C), decomposition accelerated again during summer 2003 and by September 2003 on average 85% of the *n*-alkanes were decomposed. Leaf litter degradation was also accompanied by commonly observed changes of *n*-alkane patterns. Originally high OEPs were levelled out (Fig. 1D) and long-chain *n*-alkane ratios like *n*-C₃₁/*n*-C₂₇ (Fig. 1E) and *n*-C₃₁/*n*-C₂₉ (Fig. 1F) were converging to the value 1. This observation was recently used in order to suggest and apply models that account for degradation effects when reconstructing vegetation changes using long-chain *n*-alkane ratios in soils and sediments (Zech et al., 2009a, 2010a; Buggle et al., 2010). Changing *n*-alkane patterns during degradation may reflect either preferential degradation of certain *n*-alkane homologues or addition of a soil microbial *n*-alkane pool being characterized by a different *n*-alkane pattern than leaf-waxes.

Strikingly, the mid-chain *n*-alkanes ($\sum(n\text{-C}_{20} \text{ to } n\text{-C}_{24})$), which are present not only in higher plant leaf-waxes but are known to be produced by microbial organisms (Jones, 1969; Grimalt et al., 1988; Ladygina et al., 2006), reveal different degradation patterns in comparison with the long-chain *n*-alkanes. Firstly, they occur in lower concentrations (Figs. 2 and 3A) and show much less decrease during 27 months of leaf litter degradation (from 6.1 to 4.9 $\mu\text{g/g}$ litter and mean turnover time of 34.1 months; Table 2). Secondly, the trends are often reversed when compared to the long-chain *n*-alkanes. While in spring and summer 2002 the long-chain *n*-alkanes were decomposed fastest and their decrease also accelerated again in summer 2003, the mid-chain *n*-alkane masses were increasing during these periods (Fig. 3B). Hence, we suggest that the increase in mid-chain *n*-alkanes concentrations and masses may be the result of soil microbial activity, whereas the long-chain *n*-alkanes increasingly represent a mixed pool of decomposing plant-derived *n*-alkanes and *in situ* produced microbial *n*-alkanes or externally introduced *n*-alkanes. Further evidence for questioning the long established belief that long-chain *n*-alkanes in soils predominantly derive from plants comes from the D/H isotopic signature of the individual long-chain *n*-alkanes.

3.2. Bulk litter δD -values and compound-specific δD -values of individual *n*-alkanes

Fig. 3D illustrates that bulk organic matter experienced systematic δD enrichment during leaf litter degradation especially in the summers 2002 and summer 2003 (on average from -99% to -91%). This can be caused by the preferential removal of relatively D-depleted organic compounds, and/or by the exchange of organically bound hydrogen atoms in organic compounds with reactive functional groups, such as carboxyl and hydroxyl groups (Schimmelmann et al., 2006) with D-enriched soil water.

The long-chain *n*-alkanes *n*-C₂₇, *n*-C₂₉, and *n*-C₃₁ in the broad-leaf litter species are significantly depleted in deuterium compared to bulk organic matter, with δD values of *Acer* *n*-alkanes ranging from -145% to -162% , *Fagus* from -157% to -182% and *Sorbus* from -162% to -204% (Fig. 3C). While bulk δD values show no seasonal variations, δD values of all 5 analysed individual *n*-alkanes reveal systematic variations, which are strikingly similar to those of mid-chain *n*-alkane concentrations and masses (Fig. 3A–C). According to the employed student's *t*-tests, the seasonal variations are significant on levels of (*P*-values) 0.030, 0.024, 0.115, 0.045 and 0.242 for *n*-C₂₇ and *n*-C₂₉ of *Acer*, *n*-C₂₇ of *Fagus* and *n*-C₂₉ and *n*-C₃₁ of *Sorbus*, respectively. On average, *n*-alkanes become depleted by about 12‰ from June to November 2001. In March and June 2002, when mid-chain *n*-alkanes indicate *in situ* production of microbial *n*-alkanes (Fig. 3B), mean δD values increase by about 5‰ but become more negative again in October (about 6‰). In contrast, March and September 2003 are characterized by D-enrichment (about 10‰), which is once again accompanied by an increase of the mid-chain *n*-alkanes.

Similar to the *n*-alkane patterns and masses discussed above, also the seasonal δD -variations of the investigated *n*-alkanes suggest that the long-chain *n*-alkanes do not explicitly derive from the degrading leaf litter. Even though the overall concentrations and masses of long-chain *n*-alkanes decrease during the experiment (Figs. 1B and C), our findings strongly suggest that there is an additional *n*-alkane source, which is sensitive to the seasonal δD

Fig. 1. Total litterbag mass losses (A) from Kalbitz et al. (2006) and long-chain *n*-alkane characteristics for three different leaf litter species (*Acer*, *Fagus* and *Sorbus*) during 27 months of leaf litter degradation in a field experiment. (B) *n*-Alkane concentrations, (C) *n*-alkane masses, (D) odd-over-even predominance and (E and F) *n*-alkane ratios. Bright background indicates summer, dark one winter. *Referenced against the initial *n*-alkane masses (month 0 = 100%).

variations of the precipitation ranging from about -87‰ in the winter to -18‰ in the summer.

4. DISCUSSION

4.1. Absence of D/H exchange reaction and negligible fractionation during biodegradation

Temporal shifts of bulk organic matter δD values during leaf litter decomposition and diagenesis can be explained by the preferential removal of isotopically different labile organic compounds. Furthermore, organically bound hydrogen atoms present in certain functional groups (e.g. in carboxyl and hydroxyl groups) are prone to hydrogen

exchange reactions with surrounding water (Schimmelmann et al., 2006). While these processes may account for the observed bulk δD shifts in our litterbag experiment (Fig. 3D), they are unlikely to explain the observed δD variations of individual *n*-alkanes (Fig. 3C). It is generally accepted that even over geological timescales post-depositional processes (exchange reactions) do not affect δD values of sedimentary *n*-alkanes (Yang and Huang, 2003; Sessions et al., 2004; Pedentchouk et al., 2006; Dawson et al., 2007; Jones et al., 2008; Li et al., 2009).

Another possible process, which has to be considered when searching for explanations for our *n*-alkane δD litterbag results is fractionation due to biodegradation. Pond et al. (2002) have shown in a biodegradation study

Fig. 2. *n*-Alkane concentration patterns of three different leaf litter species (*Acer*, *Fagus* and *Sorbus*) before (0 months) and after (27 months) leaf litter degradation in a field experiment.

of crude oil that due to preferential decomposition of D-depleted *n*-alkanes, remaining short-chain *n*-alkanes became D-enriched by up to $\sim 25\%$. However, the authors also reported that the D/H composition of long-chain *n*-alkanes was relatively stable. Although we cannot completely rule out a minor effect of biodegradation on our *n*-alkane δD results, fractionation cannot explain the seasonal variations, which we found during leaf litter degradation.

4.2. Possible sources of the new long-chain *n*-alkanes

In our litterbag experiment, neither the initial increase of long-chain *n*-alkanes (Fig. 1B and C) nor the intermittent increase of mid-chain *n*-alkanes (Fig. 3A and B) nor the δD variations of individual *n*-alkanes (Fig. 3C) can be explained solely by the decomposition of plant-derived leaf-

wax *n*-alkanes in the litter. Therefore, we argue that a significant pool of additional *n*-alkanes began to influence our experiment shortly after it was started. Both the quantitative role and the D/H composition of this additional *n*-alkane pool seem to depend on the season.

Recently, significant seasonal δD shifts of up to $\sim 40\%$ were reported for leaf-wax *n*-alkanes (Pedentchouk et al., 2008; Sachse et al., 2009). Furthermore, it is well known that abrasion from leaf-surfaces produces aerosols reflecting the leaf-wax lipid composition (Rogge et al., 1993; Simoneit, 2005; Andreou and Rapsomanikis, 2009). Consequently, the deposition of these aerosols on forest soils and our litterbags may partly account for the observed increases of *n*-alkane masses (Fig. 1C) and the seasonal patterns of *n*-alkane specific δD values (Fig. 3C). However, the additional input of higher plant leaf-wax lipids by aerosols

Fig. 3. (A) Mid-chain *n*-alkane concentrations ($\sum(n\text{-C}_{20}\text{ to }n\text{-C}_{24})$), (B) mid-chain *n*-alkane masses ($\sum(n\text{-C}_{20}\text{ to }n\text{-C}_{24})$), (C) compound-specific δD -values of the most abundant individual *n*-alkanes and (D) bulk δD -values for three different leaf litter species (*Acer*, *Fagus* and *Sorbus*) during 27 months of leaf litter degradation in a field experiment. Bright background indicates summer, dark one winter. *Referenced against the initial *n*-alkane masses (month 0 = 100%).

can neither explain sufficiently the mid-chain *n*-alkane increases (Fig. 3B) in times of strongest long-chain *n*-alkane decreases, nor the systematic trends of the OEPs (Fig. 1D) in our samples.

Many bacteria produce *n*-alkane distribution patterns ranging from C_{11} to C_{35} often without any OEP (Ladygina et al., 2006). This allows distinguishing between *n*-alkanes derived from bacteria and higher plant-leaf waxes. The *n*-alkane patterns of many fungi (e.g. *Aspergillus* sp.) resemble those of the bacteria. Jones (1969) and Weete (1972) reported on many soil microorganisms having no or at least low OEPs and *n*-alkane patterns maximising in the range from $n\text{-C}_{27}$ to $n\text{-C}_{31}$. Only algae seem to be characterized by high OEPs similar to higher plants (Jones, 1969). Although it may be thus very difficult to attribute them a certain OEP, all soil microorganisms together (sum of bacteria, fungi and algae) likely exhibit very low OEPs. Concentrations in fungi mycelia range from 0.06% to 0.70% of dry biomass and from 0.17% to 2.69% of dry biomass in gram-positive aerobic bacteria (Jones, 1969). *n*-Alkanes mainly of bacterial origin were for instance detected in throughfall and stem water, presumably especially in colloidal dispersion (Colina-Tejada et al., 1996). And last but not least, Grimalt et al. (1988) found that the wet storage of sediment samples produced mid-chain *n*-alkanes with no OEP.

Hence we hypothesize that a microbial *n*-alkane pool in our leaf litter samples could be responsible for the observed increases of mid- and long-chain *n*-alkane amounts, the declining OEPs and the seasonal δD variations.

4.3. Modelling leaf litter *n*-alkane decay and built-up of a microbial *n*-alkane pool – explaining the seasonality of the *n*-alkane δD results

While we argued that the plant leaf-wax *n*-alkanes in the litter have not undergone any significant isotopic shift dur-

ing biodegradation (Section 4.1), the postulated soil microbial *n*-alkane pool (Section 4.2) is not only variable in its amount over time due to its built-up and simultaneous decomposition, but also susceptible to variations in δD of precipitation and soil water, because soil microorganisms incorporate this isotopic signature during biosynthesis. In order to assess the resulting impact on δD values in decaying litter semi-quantitatively, we propose a conceptual model based on an isotope mass balance calculation (see Supplementary material).

In this model, the leaf-wax *n*-alkane decay, the built-up of a microbial *n*-alkane pool and the total *n*-alkane pool are parameters to be set up in two-month steps. Fig. 4A and B illustrate these parameters in a model run as we consider it to reflect realistic conditions. Accordingly, the decay of plant derived *n*-alkanes starts with a two-month time lag; the decomposition rate is decreasing in winter months and increasing in summer months. δD values of the plant-derived *n*-alkanes remain constant at -160‰ over the period of leaf litter degradation. We assume that there is an increase in the proportion of microbial *n*-alkanes during the first several months as well as during the following spring and early summer, when plenty of easily degradable organic compounds from fresh leaf litter are available. Slight decreases are assumed for the winter months and the year 2003 because of lower temperatures and less favourable substrate conditions. Furthermore, we estimate the proportion of newly synthesized *n*-alkanes (for each 2 month step in the model) versus ‘old’ microbial *n*-alkanes based on expected microbial activity, which is low in winter, high in spring/summer and gradually decreases from year to year (Fig. 4C). δD values of newly synthesized *n*-alkanes are estimated by assigning a D-depletion of -160‰ during biosynthesis (Sachse et al., 2006) relative to δD of the source water (Fig. 4D). We acknowledge that this assumption involves some uncertainty because D/H fractionation

Fig. 4. (A) Modelled total, plant and microbial *n*-alkane masses, (B) modelled proportion of plant versus microbial *n*-alkanes, (C) modelled proportion of newly synthesized microbial *n*-alkanes (estimated for each two month step) versus 'old' ones (already existing microbial *n*-alkanes are classified as 'old' in each model step), (D) modelled δD values for source water, newly synthesized and total microbial *n*-alkanes and (E) comparison of modelled total *n*-alkane δD values with measured δD values from the litterbag experiment. Bright background indicates summer, dark one winter. *Referenced against the initial *n*-alkane masses (month 0 = 100%).

factors for soil microorganisms are unknown and e.g. Zhang et al. (2009) reported on systematically varying D/H fractionation factors of bacteria depending on the metabolic pathway. However, given that soil fungi cells belong like plant cells to the eukaryotic cell type and hence have many metabolic pathways in common, our assumption is reasonable. δD values of total microbial *n*-alkanes were calculated from newly synthesized and 'old' *n*-alkanes using an isotope mass balance calculation (Fig. 4D). Finally, the total *n*-alkane δD values were determined by adding together the total microbial and the plant-derived *n*-alkanes using an isotope mass balance, too (Fig. 4E).

For the here presented model run, the results show the same seasonal trends as the δD mean values obtained from the litterbag experiment. Also amplitudes of δD variations are very similar (Fig. 4E). The only outlier is September 2003, where more positive measured δD values can be possibly explained by the 2003 European heat wave. This finding provides support for the idea that leaf litter decomposition is accompanied by a simultaneous build-up of a microbial *n*-alkane pool, which can cause seasonal variations of long-chain *n*-alkane δD values of up to 25‰ in the case of *n*-C₂₇ in *Fagus* litter (average value 13‰).

4.4. Implications for turnover-times, origin of long-chain *n*-alkanes in soils/sediments and δD values of *n*-alkanes as paleoclimate proxy

Seasonal δD variations of leaf-wax *n*-alkanes have already been reported (Pedentchouk et al., 2008; Sachse et al., 2009). The authors concluded that *n*-alkanes in plant leaf waxes have very short turnover times (within weeks).

For comparison, we calculated mean *n*-alkane turnover times for leaf litter decomposition of around 11.7 months (Table 2) and assumed for our model the microbial *n*-alkane pool to be renewed by 60–80% within 2 months during summer seasons. Nevertheless, we are aware that under steady-state conditions in soils, where *n*-alkanes are protected against degradation e.g. in microaggregates, turnover times will be much longer. For instance, Wiesenberg et al. (2004) reported turnover times of *n*-alkanes in cropped soils ranging from 35 to 60 years.

Sachse et al. (2009) furthermore concluded from their results that the isotopic signal reaching soils and sediments represents only the last weeks before leaf senescence. Since D-enrichment by evapotranspiration in soil and leaf-water is less pronounced in autumn compared to summer, the δD values of *n*-alkanes in leaf litter are more negative than in fresh leaves. The strong influence of D-enrichment in leaf-water due to transpiration on δD values of plant waxes was recently also demonstrated by Feakins and Sessions (2010). Accordingly, this finding can partly explain the significant D-depletion of up to 94‰ (average 55‰) observed by Chikaraishi and Naraoka (2006) for the transition from fresh leaves to soils. However, it can not explain the progressive depletion from leaf litter to mold and finally soil. The δD results from our litterbag experiment support the idea that microbial reworking during leaf litter degradation is responsible for this depletion, because soil microorganisms use soil water as source, whereas plants incorporate D-enriched leaf-water. Further studies should explore the extent of seasonal variations in δD values of *n*-alkanes in microbially active topsoils.

The *n*-alkane concentration pattern (Fig. 2), the discussion about microbial *n*-alkane sources (Section 4.2) and the modelling results of seasonal δD variations (Section 4.3.) have shown that even when *n*-alkane patterns still look very characteristic for leaf-waxes, significant amounts of *n*-alkanes can be contributed by microorganisms. Furthermore, microorganisms typically reveal high abundances of mid-chain *n*-alkanes and are also able to produce short-chain *n*-alkanes. Hence, virtually all *n*-alkanes which are used as biomarkers for terrestrial or aquatic plants in paleoclimate studies of lake sediments can potentially be influenced by early degradation as well as by eroded or leached soil organic matter. Firstly, this may help explaining *n*-alkane pattern differences between lacustrine sediments and dominant vegetation in the catchment as for instance described by Sachse et al. (2006). Secondly, these soil microbial *n*-alkane pools have more negative δD values than terrestrial plants (leaf-water D-enrichment) and at the same time can be supposed to have more positive δD values (soil water enrichment) than aquatic plants except for semi-arid and arid ecosystems like Tibetan Plateau (Mügler et al., 2008). Our results suggest that paleoclimate studies using δD values as a proxy for paleohydrology should consider not only paleovegetation history (Liu et al., 2006; Smith and Freeman, 2006; Feakins and Sessions, 2010), but also potential contribution of organic compounds from microbial biomass with different δD signature.

5. CONCLUSIONS

Aiming at contributing to the discussion whether *n*-alkane biomarkers and especially the paleoclimate proxy δD of long-chain *n*-alkanes are affected by early diagenesis/decomposition, we investigated three different broad-leaf litter species, which have been degraded in the field for 27 months. From our results and the discussion we draw several conclusions:

- Concentrations and masses of plant leaf-wax *n*-alkanes are decreasing rapidly during leaf litter decomposition (~85% in 27 months, mean turnover time around 11.7 months).
- Leaf litter degradation is accompanied by characteristic changes of the *n*-alkane patterns, namely the decrease of originally high OEPs and the convergence of long-chain *n*-alkane ratios to the value 1. This should be taken into account when trying to reconstruct vegetation changes based on *n*-alkane patterns (Zech et al., 2009a).
- Changing *n*-alkane patterns, initial long-chain *n*-alkane increases and intermittent mid-chain *n*-alkane increases may indicate that a microbial *n*-alkane pool is rapidly build up and starts to overprint the original *n*-alkane patterns of decomposing leaf litter. Admittedly, a partly contribution of leaf-wax *n*-alkanes by throughfall is very likely, too.
- The build-up of this additional *n*-alkane pool, which is susceptible to δD variations of source water, can cause seasonal δD variations in decomposing leaf litter of up to 25‰ (*Fagus*, *n*-C₂₇, average 13‰). A respective conceptual model is proposed to corroborate and visualize this idea.
- The build-up of a soil microbial *n*-alkane pool may help explaining why more negative δD values are observed in soils and sediments compared to δD values of fresh leaf litter. This should be kept in mind when applying the *n*-alkane δD paleoclimate proxy to terrestrial paleosols.
- Similarly, unless SOM erosion/leaching and early leaf litter degradation can be excluded, also δD values of long-chain *n*-alkanes in lacustrine sediments are likely to reflect a mixed plant and microbial signal rather than a solely plant leaf-wax signal. Short- and mid-chain *n*-alkanes are not exclusive biomarkers for aquatic plants either, but can be produced by soil microorganisms, too. Therefore care must be taken when interpreting δD values of sedimentary *n*-alkanes.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.gca.2011.06.006.

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