

Reply to referee 1 comments:

Referee comments are in blue.

5 Abstract:

Page 1 lines 8-10: The application of CSIA is not restricted only to freshwater systems, as many studies from marine environment (For eg., Canuel et al., 1997; Limnol Oceano, 42, 1570) also show its importance  
Added the relevant information in abstract and introduction, page 5 line 10 and 35.

10 Introduction:

Page 2 line 16: Huang et al. (1997) Punctuation- please remove comma here and many other places Page 2 line 28: I feel it should be 'fresh plant material with organic matter to the organic horizons and upper mineral soil horizon'

Removed comma page 6 line 14, page 8 line 23 and page 11 line 18,19. Restructured the sentence, page 6 line

15 26.

Carbon preference Index of alkanes can also be given in addition to average chain length.

20 CPI values were in support of our finding of general odd over even predominance in long-chain *n*-alkanes through all the degradation stages, which we have mentioned in results and discussion on Page 9 line 5. A sentence on CPI values result added on page 9 line 12.

## 2. Materials and methods

Page 3, line 13: It is better to define Oi-Oe-Oa horizons to the readers at the first time

25 Line 15: 'moder' type spelling correction

2.4 Page 4 line 7: Please define OM at the first usage Line 5: please remove the bracket for reference Schmidt et al., 1999

Oi, Oe and Oa horizons defined in study area section page 7 line 9-10. Spelling corrected. OM is defined in the introduction section. Reference corrected page 8 line 5.

30

Section 3.2 summarizes that bulk  $\delta^{13}C$  values should not be relied for sediment source attribution compared to compound specific isotope values. However the issues/reasons (other than enrichment) while considering bulk isotope values are not discussed in detail.

35 Our aim of this study is to test if isotopic signatures of tracers used for sediment source attribution are stable which requires non-fractionation during detachment and transport. Our assumption is that if they are stable during degradation, they will also be stable during detachment and transport. Thus, the continuous enrichment

in bulk  $\delta^{13}\text{C}$  values through the degradation stages is *the* reason of concluding that they are not suitable as sediment tracers. We do not know of any other major reason to reject bulk isotope analysis for sediment source attribution. Sorry, if this was not clear enough, we will have a critical look at the formulation of our aims and assumptions. We would like to add, that source apportionment with differences in  $\text{C}_3$ - and  $\text{C}_4$ - vegetation sources has been done successfully, because differences in bulk isotopic signals of sources are bigger, thus small deviations by fractionation might not substantially alter the results.

5

A relevant sentence is added to the results section page 10 line 10.

10 The manuscript is written well, but the novelty and importance of present study need to be highlighted in the manuscript.

Thank you and also for pointing out, that we should highlight our results more.

The introduction part is re-structured. In the conclusion part we added more sentences, page 12 line 8, 9, 13, and 14.

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Citation of references in the text is given alphabetical order. Normally it is cited in chronological order (ascending). Anyway please follow the journal format and modify accordingly.

20 Citations changed to chronological order.  
Inserted Upadhayay et al., (2017), see page 6 line 11 as per the suggestion.

Reply to referee 2 comments:

25 Referee comments are in blue.

#### 1. Introduction:

30 Authors have started this section by introducing the causes of fresh ecosystem degradation as result of nutrient input along with detrital riverine/ aeolian input. However, they ended up with the determination of organic matter sources and extent of degradation in a soil horizon. The connectivity between organic matter source and concerned with freshwater ecosystems has not been addressed. This aspect needs to be elaborated in the discussion or modify the introductory part for better coherence.

We modified the introduction part, please see page 5 and 6 for marked-up changes.

35

#### 2. Methodology:

A. Why inorganic carbon was not removed from the soil prior to bulk carbon isotope ratio measurement?

As the soils from forests with well-developed organic horizons are generally acidic, we expected no presence of inorganic carbon. To confirm that, we measured the pH of soils from all sites which was in the range of 3-4.2 (Page 3, line 16). Hence, it was not necessary to remove inorganic carbon from soils.

5 **B. What is the recovery % of short and long-chain compounds in the entire extraction process?**

We employed lipid extraction procedure utilizing high temperature (100°C) and high pressure (1500 psi) accelerated solvent extraction (ASE) technique. This technique has been adopted from many previous studies, which showed > 80% recovery of leaf and soil lipid biomarkers (Jansen et al., 2006; Magill et al., 2015; Ardenghi et al., 2017). We also employed 3 extraction cycles for every sample, each cycle with static hold of 10 pressure and temperature (5 min) and solvent flushing, which has been suggested to be sufficient for > 80% recovery of lipids. In our study, we did not calculate the absolute recovery of lipids from samples, however approx. 70% of an internal standard (C<sub>19:0</sub> FA) was recovered from the original amount added to test samples before lipid extraction and compound separation.

15 **C. How the methylation correction was applied for carbon isotope ratio measurement? Which formula was used and how the alcohol isotope ratio was measured?**

Correction of measured FAMES was done by mass balance for contribution of carbon added during methyl esterification to obtain the δ<sup>13</sup>C value of fatty acid using following formula;

20 
$$\delta^{13}C_{FA} = \frac{\delta^{13}C_{FAME} - (1-X)\delta^{13}C_{Methanol}}{X}$$

Where X is the ratio of carbon atoms in FA to the carbon atoms in corresponding FAME. δ<sup>13</sup>C<sub>Methanol</sub> was measured with an elemental analyser coupled to an isotope ratio mass spectrometer, using similar instrumental parameters as bulk C isotope analysis in our study. We added this information in the methods section page 9 lines 1-5.

**3. Result:**

30 **A. The best way to show the result is against the respective soil profiles. The different zone identified in the different soil profiles can also be marked for clarity.**

We modified figure 4 and 5, so that the trend of each compounds' isotope values in the horizons placed next to each other from left to right. We also defined the organic horizons (Oi-Oe-Oa) in the method section. We have also marked the horizon after which the isotopic signal remained stable with a horizontal dotted line.

35 **B. What is LB pruce\*?**

LB<sub>spruce\*</sub> is site no. 3 (Lake Baldegg catchment, Table 1) with spruce and moss as primary aboveground biomass sources and with organic horizon categorized as a raw humus. We added site abbreviations in the caption of every relevant figure for better understanding.

5 C. At many places, you are writing  $\delta^{13}\text{C}$  enrichment or depletion!! Please write an 'increase in  $\delta^{13}\text{C}$  or 'enrichment of  $^{13}\text{C}$ '.

Changed the wording in several places.

D. Why moss values are neglected? Why the significantly low  $\delta^{13}\text{C}$  values?

10 Mosses generally have lower  $\delta^{13}\text{C}$  values than higher plants as they grow in wet and humid conditions and experience less water loss due to evapotranspiration. We did not neglect mosses as we did analyze their isotopic signatures. However, regarding the overall contribution to the humus layer material, we considered spruce to be a dominant source of biomass/lipids to the soils compared to moss at site LB<sub>spruce\*</sub>. Thus, for the calculation of magnitude of change in  $\delta^{13}\text{C}$  value from fresh plant biomass to the mineral soil ( $\Delta_1$ ), only needle isotope values  
15 were considered to obtain the  $\delta^{13}\text{C}$  (fresh plant biomass) at site LB<sub>spruce\*</sub>. This was done to avoid overestimation of change/increase in  $\delta^{13}\text{C}$ , as moss isotope values are considerably depleted compared to spruce needle values. Also, isotopic composition of soils is controlled by the amount of biomass from each source on a larger timescale.

20 4. Discussion:

A. Why the bulk  $\delta^{13}\text{C}$  and is showing  $^{13}\text{C}$  enrichment? Compared to the nC28 and nC29 B. Is the true for other long-chain fatty acids and alkanes as well?

25 A+B. The majority of the bulk organic matter is considered to be less recalcitrant and more readily available to soil organisms than long-chain alkanes and fatty acids. During degradation/mineralisation, bacteria (or rather the respective enzymes) have generally a higher affinity towards the lighter isotope, which will be preferentially fed on. Hence, we observed generally a greater enrichment in  $^{13}\text{C}$  of the remaining bulk organic matter across different degradation stages.

30 A second aspect is, that previous studies have shown that lipid biomolecules are depleted in  $^{13}\text{C}$  compared to bulk organic matter due to the kinetic isotope effects during various biochemical reactions in lipid biosynthesis (Collister et al., 1994; Chikaraishi et al., 2004). Hence, we already observe lower  $\delta^{13}\text{C}$  values in long-chain fatty acids and *n*-alkanes compared to the bulk  $\delta^{13}\text{C}$  values (Page 6, line 7-8) before degradation even starts with fractionation processes.

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# Understanding the effects of early degradation on isotopic tracers: implications for sediment source attribution using compound-specific isotope analysis (CSIA)

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**Abstract.** Application of compound-specific isotope analysis (CSIA) in sediment fingerprinting source apportionment studies is becoming more frequent, as it can potentially provide robust land-use based source attribution of suspended sediments in freshwater and marine systems. Isotopic tracers such as  $\delta^{13}\text{C}$  values of vegetation-derived organic compounds are considered to be suitable for CSIA based fingerprinting method. However, a rigorous evaluation of tracer conservativeness in terms of the stability of isotopic signature during detachment and transport of soil during erosion process is essential for the suitability of the method. With the aim to identify potential fractionation and shifts in tracer signature during early degradation of organic matter in surface soils, we measured concentrations and  $\delta^{13}\text{C}$  values of long-chain fatty acids and *n*-alkanes from fresh plant biomass (as vegetation is a direct source of these compounds to the soils), degraded organic horizon (O horizon) as well as mineral soil (A horizon) from various forest types with different humus forms (five sites). The bulk  $\delta^{13}\text{C}$  values showed continuous  $^{13}\text{C}$  enrichment through the degradation stages from fresh plant material to the O and A horizon, ranging between 3.5 and 5.6‰. Compound-specific  $\delta^{13}\text{C}$  values showed a general  $^{13}\text{C}$  enrichment for both, long-chain fatty acids (up to 5‰) as well as *n*-alkanes (up to 3.9‰) from fresh plant biomass to the O horizon overlying the A horizon. However, only slight or no further changes occurred from the O to the A horizon. We also compared compound-specific  $\delta^{13}\text{C}$  values between two soil particle-size classes (< 2 mm and < 63  $\mu\text{m}$ ) from four sites and found no significant differences of tracer values between them, with even less fractionation for the long-chain *n*-alkanes within the soil particle fractions, which points to the conclusion that sampling and analysing bulk soil material might be valid for the isotopic tracer applications. We further conclude, that our results support the suitability of studied isotopic tracers as representative source soil signature in CSIA based sediment source attribution, as they demonstrated necessary stability in plant-soil system during organic matter degradation.

## 1 Introduction

Soils play a key role in the storage of organic carbon in terrestrial ecosystems (Schmidt et al., 2011; Scharlemann et al., 2014). Plant lipids constitute a major part of soil organic matter (OM) and can also be used as biomarkers for tracing plant derived OM in soil. These plant lipids are continuously transferred into the soil after their production, through litter deposition and abrasion of cuticular waxes (Glaser, 2005; Jansen and Wiesenberg, 2017). Compound-specific isotope analysis (CSIA) of lipid constituents such as long-chain fatty acids and *n*-alkanes has been routinely used as a valuable tool to infer soil OM source and turnover (Wiesenberg et al., 2004; Zocatelli et al., 2012), reconstruction of paleoenvironments and paleovegetation (Glaser and Zech, 2005; Eglinton and Eglinton, 2008), and to fingerprint sediment-bound OM sources in freshwater and marine environments (Canuel et al., 1997; Galy et al., 2011). As freshwater ecosystems are currently under the threat of high sediment input due to the intensification of anthropogenic activities and associated land-use

changes, the CSIA technique has recently been adopted for improved quantification of the sediment sources from different land-uses (Gibbs, 2008).

Sediment fingerprinting based on CSIA utilises the vegetation-specific isotopic signatures of the lipid biomarkers, to discriminate the sediment sources by the fraction attributable to the major land-uses present in the catchment. In particular, carbon isotopic composition ( $\delta^{13}\text{C}$  values) of individual long-chain fatty acids (Gibbs, 2008; Blake et al., 2012; Hancock and Revill, 2013; Alewell et al., 2016; Brandt et al., 2016; Mabit et al., 2018), long-chain *n*-alkanes (Seki et al., 2010; Cooper et al., 2015; Chen et al., 2016) and plant family-specific biomarkers (e.g., triterpenyl acetates produced by Asteraceae) (Lavrieux et al., 2019) has been used to trace freshwater sediments. CSIA technique is also complimented by the use of molecular proxies based on the concentration of these compounds such as the average chain length (ACL) values and carbon preference index (CPI) (Cooper et al., 2015). An overview of current understanding and progress of CSIA based sediment source attribution has been summarised by Reiffarth et al. (2016) and Upadhayay et al. (2017).

The vegetation specificity in isotopic composition of plant lipid components is an important property for CSIA based sediment source attribution. This specificity generally depends on biological (i.e., photosynthesis) and environmental factors (i.e., water stress) that play a role during lipid synthesis and is subsequently transferred to the soil. Huang et al.

(1997) reported long-term degradation and diagenesis processes did not result in the changes of carbon isotope composition of *n*-alkanes in agricultural soils after their transfer from fresh plant material. The  $\delta^{13}\text{C}$  signature of organic compounds does not change by volatilization, dilution, dispersion and sorption (Blessing et al., 2008 and references therein). However, large uncertainty is also observed in the isotopic stability of these tracers in the plant-soil systems (Chikaraishi and Naraoka, 2006; Nguyen Tu et al., 2011). Some studies report several per mil increase in *n*-alkane  $\delta^{13}\text{C}$  values of the soils compared to the dominant higher terrestrial plants (Ficken et al., 1998; Nguyen Tu et al., 2004; Wiesenberg et al., 2004; Griepentrog et al., 2016). Hence the effects of early degradation on these plant-derived chemical assemblages across the soil horizons, in terms of their carbon isotope ratios and molecular distributions need to be understood in greater detail. The degradation stage in the surface soils at which the tracer signature becomes stable and shows no further changes need to be identified, not only for a reliable identification of source signature for CSIA based sediment fingerprinting technique, but also for an assessment of the relative stability of the isotopic signatures during detachment and transport of sediments. Our approach is to compare original C isotopic signature from fresh plant material to the OM from the organic horizons and upper mineral soil horizon (e.g., increasing degradation stages along the organic horizons – top-soil profile). Also, it has been shown that within the soil particle-size fractions and density fractions, the stability and composition of the tracer signal may change (Cayet and Lichtfouse, 2001; Quéneá et al., 2006; Griepentrog et al., 2016). In previous CSIA based fingerprinting studies, the silt-clay fraction ( $< 63 \mu\text{m}$ ) or  $< 100 \mu\text{m}$  fraction has been chosen to extract the representative source signature (Gibbs, 2008; Blake et al., 2012; Hancock and Revill, 2013; Cooper et al., 2015). The bulk soil fraction ( $< 2 \text{mm}$ ) has also been used (Alewell et al., 2016).

We measured carbon isotopic composition and concentration of long-chain fatty acids and *n*-alkanes from fresh leaves, needles, mosses, degraded organic horizons and mineral soil (Oi-Oe-Oa-A horizons) from various forest types with different humus forms, in order to (1) understand the effect of early degradation on the isotopic tracer signals employed in CSIA based sediment fingerprinting technique and (2) compare the two biomarker classes in terms of their  $\delta^{13}\text{C}$  values and molecular distribution in the plant-soil system for potential suitability as representative source soil signature for sediment fingerprinting. We also analysed the compound-specific  $\delta^{13}\text{C}$  values from two soil particle-size fractions ( $< 2 \text{mm}$  and  $<$

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63  $\mu\text{m}$ ) to (3) understand the [soil particle](#) size dependent isotopic fractionation and its implication for [CSIA based](#) sediment source attribution.

## 2 Materials and methods

### 2.1 Study area

5 For studying the degradation effect on the vegetation-specific tracers, five sampling sites were selected (Fig. 1); of which two are in the Southern Black Forest (Germany), two in the Lake Baldegg catchment (Switzerland) and one in the Upper Sûre Lake catchment (Luxembourg). The broadleaved forest site in the Southern Black Forest (site 1; BFbeech) was mainly stocked with beech (*Fagus sylvatica*), under which a moder (duff mull) type humus with clearly separable Oi ([undecomposed OM](#)) and Oe ([partly decomposed OM](#)) horizons, but a diffused boundary between O and A horizon has developed [due to the absence of a separate Oa \(decomposed OM, humus layer\) horizon](#). The second site in Southern Black Forest is a coniferous forest where the sole tree species is spruce (*Picea abies*, site 2a; BFspruce) and a thick cover of moss (*Sphagnum quinquefarium*, site 2b; BFmoss) is growing under very acidic conditions (pH  $\sim$ 3) characterised by the development of raw humus with an easily separable sequence of Oi-Oe-Oa and A horizons. One of the two sites in the Lake Baldegg catchment has mull type humus with a thin Oi layer on top of the A layer (Site 4; LBSpruce). The mixed-forest site in the Upper Sûre Lake catchment has oak (*Quercus robur*) and spruce (*Picea abies*) cover with moder type humus (site 5; LXmixed). All sampling sites have acidic soils with pH values in the range of 3-4.2. As we are aware that a range of soil pH values might have been preferable, fully developed organic horizons are not found in slightly acidic or alkaline soils.

### 2.2 Sampling

20 Fresh leaves, needles and mosses were collected in autumn. Multiple leaves/needles (15-20) from different directions at ca. 3 m above ground were collected from an individual tree and mixed to form a sample of fresh material. The O horizon was sampled and sub-horizons within it were separated according to the humus form observed. Underlying A horizon was sampled to a maximum depth of 5 cm. Each sample was formed from a composite mixture of 3 sub-samples taken at a distance of ca. 1 m from each other.

### 2.3 Sample preparation and bulk isotope analysis

25 Collected samples were oven-dried at 40°C for 72 h. Soils were dry sieved to a particle size  $< 2$  mm and macroscopic plant remains and stones were removed with tweezers. All the samples were roughly ground with a mortar and pestle. An aliquot of ground samples was finely powdered with a ball mill (Retsch MM400, Retsch GmbH, Haan, Germany) for 90 s at a frequency of 24 s  $\text{min}^{-1}$  for analysis of bulk carbon concentration and isotopic ( $\delta^{13}\text{C}$ ) composition. Powdered samples were weighed in tin capsules and subsequently stable carbon isotope ratios ( $\delta^{13}\text{C}_{\text{bulk}}$ ) and C content were measured with an elemental analyser coupled to an isotope ratio mass spectrometer (EA/IRMS) (Sercon Integra2, Sercon Ltd., Crewe, UK). The carbon isotope values of samples are reported in a delta notation relative to the V-PDB standard. The instrumental standard deviation is lower than 0.1‰ for  $\delta^{13}\text{C}$ .

#### 2.4 Particle size separation

The particle fraction of size < 63  $\mu\text{m}$  was separated from four A horizon soil samples collected from various forest types (beech forest (site 1), coniferous forest (site 2a), coniferous forest with moss cover (site 3) and mixed forest (site 5)). Prior to the separation, soil aggregates were disrupted by ultrasonic dispersion using an ultrasonic probe (Branson 250 model, Branson Ultrasonics, Danbury, CT, USA). A 500  $\text{J ml}^{-1}$  ultrasonic energy was applied as described in Schmidt et al. (1999). This energy is sufficient for total disruption of aggregates without redistribution and compositional changes in OM. After disruption, the fraction of size < 63  $\mu\text{m}$  was separated by wet sieving and water was removed by sedimentation and centrifugation.

#### 2.5 Total lipid extraction and compounds separation

Powdered samples were extracted using an accelerated solvent extraction system (ASE 350, Dionex Corp., Sunnyvale, CA, USA) with dichloromethane (DCM)/methanol (MeOH) (9:1, v/v) over three extraction cycles at 100°C. The total lipid extract (TLE) was volume reduced by vacuum evaporation (Rocket Evaporator, Genevac Ltd., Ipswich, UK). The TLE was subsequently separated into neutral, acidic and polar fraction by solid-phase extraction using aminopropyl-bonded silica gel as stationary phase. The acidic fraction (including free fatty acids) was methylated at 60°C for 1 h using 1 mL of 12–14% boron trifluoride ( $\text{BF}_3$ ) in MeOH and fatty acid methyl esters (FAMES) were extracted with hexane and 0.1 M KCl. The aliphatic hydrocarbons containing long-chain *n*-alkanes were separated from neutral fraction obtained earlier using a column filled with an activated silica gel by eluting with *n*-heptane.

#### 2.6 Biomarker analysis

Individual compounds in the FAMES and aliphatic hydrocarbon fractions were identified using a TRACE GC Ultra gas chromatograph coupled with a DSQ II mass spectrometer (MS) (Thermo Scientific, Waltham, MA, USA). Quantification was performed using the same GC coupled with a flame ionization detector (FID) (Thermo Scientific, Waltham, MA, USA). The GC method was set identical during both measurements as described in Alewell et al. (2016).

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#### 2.7 Compound-specific isotope analysis (CSIA)

The  $\delta^{13}\text{C}$  isotopic composition of individual FAMES and *n*-alkanes was determined using a Trace 1310 GC with split/splitless injector and FID detector interfaced on-line via a GC-IsoLink II to a ConFlo IV and Delta V Plus isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany). A DB-5MS capillary column (50  $\times$  0.2 mm i.d., 0.33  $\mu\text{m}$  film thickness, J & W Scientific, Folsom, CA, USA) was used. The GC temperature program for *n*-alkanes was 70°C (held 4 min) to 320°C (held 35 min) at 5°C  $\text{min}^{-1}$ . The GC temperature program for FAMES was 70°C (held 4 min) to 150°C at 20°C  $\text{min}^{-1}$  and afterwards to 320°C (held 40 min) at 5°C  $\text{min}^{-1}$ . He (purity 5.0) was used as a carrier gas at a constant flow of 1  $\text{ml min}^{-1}$ .  $\text{CO}_2$  of known  $\delta^{13}\text{C}$  isotopic composition used as reference gas was automatically introduced via a ConFlow IV into the isotope ratio mass spectrometer in a series of 5 pulses at the beginning and 4 pulses at the end of each analysis during every measurement. The system was externally calibrated with standard mixtures A6 for *n*-alkanes (*n*- $\text{C}_{16}$  to *n*- $\text{C}_{30}$ ) and F8-3 for FAMES (even chain fatty acid methyl and ethyl esters from *n*- $\text{C}_{14:0}$  to *n*- $\text{C}_{20:0}$ ), both obtained from Arndt Schimmelmann, Indiana University, IN, USA. Isotopically certified *n*- $\text{C}_{24:0}$ , *n*- $\text{C}_{30:0}$  (Arndt Schimmelmann, Indiana University, IN, USA) and *n*- $\text{C}_{26:0}$ , *n*- $\text{C}_{28:0}$  (Sigma-Aldrich, St. Louis, MO, USA) FAMES were further added to the F8-3 mixture. The carbon isotope values of samples are reported in delta notation relative to the standard V-PDB, averaging at



least three replicate measurements. [The analytical uncertainty is lower than ±0.5‰](#). The reported  $\delta^{13}\text{C}$  values of FAMES were corrected for the additional carbon atom introduced during methylation [using the following formula](#):

$$\delta^{13}\text{C}_{\text{FA}} = \frac{\delta^{13}\text{C}_{\text{FAME}} - (1-X)\delta^{13}\text{C}_{\text{Methanol}}}{X}$$

Where X is the ratio of C atoms in the FA to the C atoms in the corresponding FAME.  $\delta^{13}\text{C}_{\text{Methanol}}$  was measured with an elemental analyser coupled to an isotope ratio mass spectrometer (EA/IRMS), using similar instrumental parameters as the bulk C isotope analysis described above.

### 3 Results and discussion

#### 3.1 Concentration of biomarkers

The lipids from fresh leaves as well as O and A horizons showed even-over-odd predominance in long-chain fatty acids ( $> n\text{-C}_{22:0}$ ) and odd-over-even predominance in *n*-alkanes ( $> n\text{-C}_{25}$ ), a property usually ascribed to the higher-plant biomass derived lipids (Eglinton et al., 1962; Kolattukudy et al., 1976; Amblès et al., 1994a). [To quantify this pattern of the chain length preference, carbon preference index \(CPI\) values were also calculated](#) (Eglinton et al., 1962). [Mean CPI values of fatty acids \( \$n\text{-C}\_{22:0} - 30:0\$ \) and \*n\*-alkanes \( \$n\text{-C}\_{23-33}\$ \) were 5.7 and 5.5 respectively, indicating the input from higher plants](#). Similar concentrations (relative abundance with regard to total OM) of both of the compound classes were observed between fresh plant biomass and degraded OM, with slight increase within O horizon (Fig. 2). We defined concentration in mineral soil also with regard to total soil OM and not to soil weight for its better comparability to the fresh plant biomass and O horizon. We found a strong increase in compound concentrations in the mineral soils collected from various sites (Fig. 2), except site BF<sub>moss</sub>. The strong increase in the concentrations from O horizon to mineral soil (A horizon) indicates a preferential preservation of long-chain fatty acids and *n*-alkanes compared to soil bulk OM (Fig. 2). In general, long-chain fatty acids were relatively more abundant than *n*-alkanes in the plant–soil system, similar to previously reported findings (van Bergen et al., 1998; Naafs et al., 2004; Chikaraishi and Naraoka, 2006).

Interspecies variability was recorded by the molecular distribution patterns of the compounds in fresh plant biomass (Fig. S1a and b). The coniferous trees had relatively high concentrations of *n*-C<sub>29</sub> alkane and *n*-C<sub>24:0</sub> fatty acid. Fresh leaves of broadleaved trees showed relatively high concentrations of *n*-C<sub>27</sub> alkane and *n*-C<sub>28:0</sub> fatty acid. The *sphagnum* species were characterised by high *n*-C<sub>23</sub> or *n*-C<sub>21</sub> alkane, differing from conifers and broadleaved trees, also consistent with previously reported distribution pattern (Maffei et al., 2004; Bush and McInerney, 2013).

However, molecular distribution patterns observed in fresh plant biomass were not necessarily transferred to the O and A horizons (Fig. S1a and b). This was shown by the changes in average chain length (ACL) values of both compound classes (Fig. 3). Relatively higher ACL values of *n*-alkanes in the A horizon compared to overlying horizons from some of the sites might have been the result of preferential degradation of shorter chained homologues ( $< n\text{-C}_{27}$ ) and preservation of longer chained homologues from *n*-C<sub>27</sub> and higher (Lichtfouse et al., 1998; van Bergen et al., 1998; Marseille et al., 1999). In case of long-chain fatty acids, changes in the molecular distribution patterns (Fig. S1a) and decrease in the ACL values (Fig. 3) from fresh matter to the A horizon was observed at most sites. This might be explained by the production of long-chain fatty acids ( $> n\text{-C}_{20:0}$ ) in O and A horizon from the oxidation of other components of vegetation-derived lipids such as *n*-alkanes and *n*-alkanols (Amblès et al., 1994a, 1994b; Marseille et al., 1999) and also a contribution of shorter chained homologues from microorganisms (Lichtfouse et al., 1995; Chikaraishi and Naraoka, 2006).

### 3.2 Stable carbon isotopic composition ( $\delta^{13}\text{C}$ values)

The bulk stable carbon isotope values were in between -32.4‰ and -26.1‰, similar to the typical  $\delta^{13}\text{C}$  values of  $\text{C}_3$  plant-soil system (Diefendorf et al., 2010). During degradation, the carbon isotopic composition generally showed consecutive enrichment in  $^{13}\text{C}$  from fresh plant biomass (leaves, needles and mosses) via organic horizons to the mineral soil. The  $\delta^{13}\text{C}$  increment from fresh plant biomass to the A horizon varied between 3.5‰ ( $\text{BF}_{\text{beech}}$ ) and 5.6‰ ( $\text{BF}_{\text{moss}}$ ) (Fig. 4). Selective degradation of bulk OM with depleted carbon isotopic signature has been observed previously, but with less significant changes probably related to short timescales (Nguyen Tu et al., 2004). Our findings report consistent degradation (with  $p$ -value = 0.72 denoting similar extent of degradation across studied forest systems) as well as consecutive increase in  $\delta^{13}\text{C}$  values through degradation stages ( $p$ -value < 0.001 denoting significantly different  $\delta^{13}\text{C}$  values). Hence the cautious use of bulk carbon isotope values for using it as source soil signatures is necessary, especially when the vegetation sources found are either  $\text{C}_3$  or  $\text{C}_4$  only.

Compared to bulk isotopic composition of fresh plant biomass, compound-specific isotope values of long-chain fatty acids and  $n$ -alkanes were generally lower by -3‰ and -3.7‰ respectively (Fig. 4 and 5), in agreement with previous studies (Collister et al., 1994; Huang et al., 1997; Nguyen Tu et al., 2011). The compound-specific  $\delta^{13}\text{C}$  values of long-chain fatty acids ( $n\text{-C}_{24:0}$  to  $n\text{-C}_{30:0}$ ) and  $n$ -alkanes ( $n\text{-C}_{25}$  to  $n\text{-C}_{31}$ ) showed general increase in  $\delta^{13}\text{C}$  values from fresh plant biomass to the O horizon overlying the mineral soil (A horizon), however only slight or no further changes to the A horizon (Fig. 5a-h, Table 2). Both the compound classes showed similar trends of  $^{13}\text{C}$  enrichment at each site. Exceptionally, some  $n$ -alkane homologues showed a strong enrichment between O and A horizon, e.g.,  $\text{BF}_{\text{moss}}$  ( $n\text{-C}_{25:27}$ ),  $\text{LB}_{\text{spruce}}$  ( $n\text{-C}_{29}$ ) and  $\text{LX}_{\text{mixed}}$  ( $n\text{-C}_{27,29}$ ) sites. Fresh moss material exhibited the lowest  $\delta^{13}\text{C}$  values for both the compound classes, -37.7‰ ( $n\text{-C}_{26:0}$  FA) and -40.4‰ ( $n\text{-C}_{25}$  alkane) of *Sphagnum quinquefarium* ( $\text{BF}_{\text{moss}}$ ) and -39.4‰ ( $n\text{-C}_{26:0}$  FA) and -37‰ ( $n\text{-C}_{27}$  alkane) of *Thuidium tamariscinum* ( $\text{LB}_{\text{spruce}}$ ), similar to previously reported sphagnum  $n$ -alkane isotope values (Brader et al., 2010).  $\delta^{13}\text{C}$  values of mosses are lower as they generally grow in wet and humid conditions and experience less water loss due to evapotranspiration (Sachse et al., 2006). Sites 2a ( $\text{BF}_{\text{spruce}}$ ) and 2b ( $\text{BF}_{\text{moss}}$ ) both are located in a spruce forest with thick moss cover. Hence the strong isotopic enrichment in  $n\text{-C}_{25}$  and  $n\text{-C}_{27}$  alkane (3.9‰ and 2.4‰ respectively) observed from O horizon to mineral soil (A horizon) at  $\text{BF}_{\text{moss}}$  might have caused by significantly higher long-term input from spruce (with comparatively higher  $\delta^{13}\text{C}$  value) compared to the fresh and degraded moss to the mineral soil. However, supportive data of, e.g., long-term biomass inputs to soil from different species present at the particular site, was not available. Also site  $\text{LX}_{\text{mixed}}$  has a vegetation cover of two distinct species (table 2). The difference in isotopic composition of fresh plant biomass of these two species along with its seasonal variability might be a cause of changes in  $\delta^{13}\text{C}$  values during degradation. Also, isotopically distinct microbial input of long-chain compounds cannot be excluded (Marseille et al., 1999; Chikaraishi and Naraoka, 2006).

At sites where single dominant vegetation is present, we observed increase up to 5‰ in long-chain fatty acids and up to 3.2‰ in long-chain  $n$ -alkanes from fresh plant biomass to A horizon, similar to the previous findings (Chikaraishi and Naraoka, 2006; Griepentrog et al., 2015, 2016). It was found that most of the degradation effect on isotopic composition took place up to the O horizon and slight or no further changes were observed during transfer to the A horizon from the overlying O horizon. Table 2 summarizes this finding where we compared the changes in compound-specific isotope values with the level of significance. We calculated the magnitude of enrichment/depletion in  $^{13}\text{C}$  from mineral soil horizon with respect to fresh biomass ( $\Delta_1$ ), and also with respect to the overlying O horizon ( $\Delta_2$ ) as follows:

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$$\Delta_1 = \delta^{13}C_{(A \text{ horizon})} - \delta^{13}C_{(\text{fresh plant biomass})}$$

$$\Delta_2 = \delta^{13}C_{(A \text{ horizon})} - \delta^{13}C_{(\text{overlying O horizon})}$$

For the calculation of  $\Delta_1$ , we excluded the  $\delta^{13}C$  values of fresh moss to obtain  $\delta^{13}C$  (fresh plant biomass) value, only at site 3. This was done to eliminate the overestimation of the magnitude of isotopic change, as mosses had considerably low isotope values compared to other fresh materials (Fig. 5).

The bulk  $\delta^{13}C$  values showed continuous increase from fresh plant material through the degradation stages to the A horizon and did not exhibit the stability needed for the use as isotope tracer. Furthermore, fresh plant biomass generally exhibited a wider range of compound-specific  $\delta^{13}C$  values (7.5‰ for *n*-C<sub>25</sub> alkane and 3.9‰ for *n*-C<sub>26:0</sub> fatty acid) than bulk isotope values (1.6‰) across all the sites. Hence in terms of the stability during early degradation in soils and interspecies variability, our results confirm that the compound-specific isotope analysis is more useful technique for sediment source attribution and the analysis should not rely on bulk isotope values.

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### 3.3 Compound-specific isotopic composition of soil particle-size fractions

We compared the compound-specific  $\delta^{13}C$  values of long-chain fatty acids and *n*-alkanes from the bulk soil (< 2 mm) to the fine silt plus clay fraction (< 63  $\mu\text{m}$ ) (Fig. 6). None of the fatty acids showed a significant difference in their  $\delta^{13}C$  values between the two soil particle size fractions. Only for LB<sub>spruce</sub>\* site,  $\delta^{13}C$  values of long-chain fatty acids were considerably (up to 2.1‰), but not significantly different among the studied fractions. Similar results were obtained for long-chain *n*-alkanes, where none of the *n*-alkanes from studied sites differed significantly between the different particle size fractions, with difference of up to 1‰ (Fig. 6). Cayet and Lichtfouse (2001) reported preferential input of long-chain *n*-alkanes in soils via larger (200-2000  $\mu\text{m}$ ) particle-size fraction. However in terms of isotopic composition of soils, Qu  n  a et al. (2006) reported less than 1‰ difference in long-chain *n*-alkanes  $\delta^{13}C$  among the soil size fractions. In accordance with previous findings on *n*-alkanes, our results simultaneously studying long-chain fatty acids as well as *n*-alkanes, confirm that the compound-specific carbon isotope values of both compound classes were not significantly different between the two particle-sizes. Long-chain *n*-alkanes were found to be even less prone to the isotopic fractionation within the soil particle-size fractions. These results indicate that the analysis of bulk soil material instead of the fine soil fraction (< 63  $\mu\text{m}$ ) might be suitable for sediment source attribution from soils to freshwater systems, which might not only increase work and cost effectiveness of the method but also reduce potential error sources due to particle size fractionation effects. In general, above results further strengthen the necessary requirement of conservativeness of tracer signature during erosion processes like detachment, dispersion and sediment transport, for CSIA based sediment source apportionment.

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### 4 Conclusions

We tested the potential stability of compound-specific carbon isotope values of long chain fatty acid and *n*-alkanes during early degradation in the soil; usually employed as tracers in compound-specific isotope analysis based sediment source fingerprinting technique. We assumed that a potential stability of a tracer signature during degradation stages from fresh plant material to the mineral soil might indicate its suitability for detachment and transport processes during soil erosion. We determined a clear increase in  $\delta^{13}C$  values for both compound classes from aboveground plant biomass to the O horizon overlaying the mineral soil (A horizon), however only slight or no further changes were observed between the O and A

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horizon. The latter was in clear contrast to bulk  $\delta^{13}\text{C}$  values, which continued to become enriched in  $^{13}\text{C}$  through all degradation stages from fresh plant biomass to soil mineral horizon.

We conclude that this finding emphasizes the suitability of compound-specific isotope analysis compared to the bulk  $\delta^{13}\text{C}$  analysis for sediment fingerprinting source attribution using stable carbon isotope. The finding was consistent through the various forest types with different vegetation and humus forms studied. The degree of variability in compound-specific carbon isotopic signatures in the plant-soil system was mostly similar between both compound classes (long-chain fatty acids and *n*-alkanes). [Beyond sediment source attribution, this study also suggests necessity for careful evaluation of bulk and compound-specific isotope values for reconstruction of past C<sub>3</sub>-C<sub>4</sub> vegetation changes in soils.](#) Comparing the tracer signatures between bulk soil material and the fine soil particle-size fraction (< 63  $\mu\text{m}$ ), the long-chain *n*-alkanes were found to be less prone to the particle-size dependent isotopic fractionation than fatty acids, however none of the compounds showed significant differences between studied particle sizes. [Hence](#), we suggest that our results indicate that the bulk soil (< 2 mm) can be safely used to determine tracer signatures in CSIA based sediment source fingerprinting, irrespective of particle-size of sediments collected. [This is particularly important as isotope values could also be considered to be not affected by the particle size sorting during sediment transport in a stream.](#) The 10-fold higher concentrations of the long-chain fatty acids compared to *n*-alkanes in surface soils suggests a better analytical certainty in extraction and measurements of fatty acids in soils and sediments with low lipid content, but the joint investigation of fatty acids and *n*-alkanes would add an additional component in CSIA based sediment source fingerprinting and thus more robust results can be expected, if analytically reliable quantity of *n*-alkanes is extracted from samples.

#### Data availability

Data from isotopic and molecular measurements is available at <https://doi.org/10.5281/zenodo.3228446>.

#### Author contributions

PH supported in designing the study, carried out the sampling, lab work, biomarker quantification and isotope analyses, data evaluation, and performed the writing of the manuscript; GW assisted in analytical method development, isotope analyses, data evaluation and interpretation, and contributed to the writing; AB provided support for isotope analyses, data interpretation, and contributed to the writing; CA primarily conceptualised the study, provided support for sampling, data interpretation, and contributed to the writing.

#### Competing interests

The authors declare that they have no conflict of interest.

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**Table 1: Main characteristics of the investigated sites.**

Site area	Forest type	Site/sub site No.	Name	Dominant vegetation cover	Humus form	Soil type	MAT (°C)	MAAP (mm)	Altitude (m.a.s.l.)
Southern Black Forest	Beech forest	Site 1	BF <sub>beech</sub>	<i>Fagus sylvatica</i> (beech)	Moder	Stagnosol	8.8	1155	535
	Coniferous forest	Site 2a	BF <sub>spruce</sub>	<i>Picea abies</i> (spruce)	Raw humus	Podzol	3.9	1800	965
		Site 2b	BF <sub>moss</sub>	<i>Sphagnum quinquefarium</i> (moss)	Raw humus	Podzol	3.9	1800	965
Lake Baldegg catchment	Coniferous forest	Site 3	LB <sub>spruce*</sub>	<i>Picea abies</i> (spruce), <i>Thuidium tamariscinum</i> (moss)	Raw humus	Cambisol	8.4	1100	608
	Coniferous forest	Site 4	LB <sub>spruce</sub>	<i>Picea abies</i> (spruce)	Mull	Cambisol	8.4	1100	573
Upper Sûre Lake catchment	Mixed forest	Site 5	LX <sub>mixed</sub>	<i>Picea abies</i> (spruce), <i>Quercus robur</i> (oak)	Moder	Cambisol	9.2	970	429

Abbreviations: Black Forest (BF), Lake Baldegg (LB), Upper Sûre Lake catchment-Luxembourg (LX).

**Table 2: Range of compound-specific  $\delta^{13}\text{C}$  values between the A horizon and fresh plant biomass average ( $\Delta_1$ ), and between the A horizon and overlying O horizon ( $\Delta_2$ ) (Significance levels derived from an Anova/Tukey's HSD Test for the difference in  $\delta^{13}\text{C}$  values, given in parentheses).**

Site Name	Change in $\delta^{13}\text{C}$ value (‰)	Fatty acids				<i>n</i> -Alkanes			
		<i>n</i> -C <sub>24:0</sub>	<i>n</i> -C <sub>26:0</sub>	<i>n</i> -C <sub>28:0</sub>	<i>n</i> -C <sub>30:0</sub>	<i>n</i> -C <sub>25</sub>	<i>n</i> -C <sub>27</sub>	<i>n</i> -C <sub>29</sub>	<i>n</i> -C <sub>31</sub>
BF <sub>beech</sub>	$\Delta_1$	4.0 (***)	3.6 (**)	3.0 (**)	1.7 (n.s.)	3.1 (**)	3.2 (**)	2.9 (**)	-
	$\Delta_2$	0.9 (n.s.)	0.4 (n.s.)	0.8 (n.s.)	-1.9 (n.s.)	0.2 (n.s.)	-0.1 (n.s.)	-0.9 (n.s.)	-
BF <sub>spruce</sub>	$\Delta_1$	5.0 (*)	1.8 (n.s.)	0.2 (n.s.)	0.2 (n.s.)	0.9 (n.s.)	0.6 (n.s.)	1.1 (n.s.)	-
	$\Delta_2$	0.0 (n.s.)	-0.7 (n.s.)	-0.3 (n.s.)	-0.2 (n.s.)	-0.3 (n.s.)	0.8 (n.s.)	0.5 (n.s.)	-
BF <sub>moss</sub>	$\Delta_1$	5.2 (*)	4.6 (n.s.)	1.3 (n.s.)	-0.3 (n.s.)	7.8 (**)	5.3 (*)	1.7 (n.s.)	1.7 (n.s.)
	$\Delta_2$	0.3 (n.s.)	1.1 (n.s.)	0.7 (n.s.)	-0.3 (n.s.)	3.9 (n.s.)	2.4 (n.s.)	1.4 (n.s.)	0.6 (n.s.)
LB <sub>spruce*</sub>	$\Delta_1$	3.1 (n.s.)	0.6 (n.s.)	-0.5 (n.s.)	-1.5 (n.s.)	2.3 (n.s.)	1.6 (n.s.)	0.5 (n.s.)	-
	$\Delta_2$	0.8 (n.s.)	1.0 (n.s.)	0.3 (n.s.)	0.2 (n.s.)	0.5 (n.s.)	-0.3 (n.s.)	-1.3 (n.s.)	-
LB <sub>spruce</sub>	$\Delta_1$	3.8 (n.s.)	1.6 (n.s.)	0.0 (n.s.)	-0.4 (n.s.)	-	-	-	-
	$\Delta_2$	2.0 (n.s.)	1.9 (n.s.)	-0.7 (n.s.)	-1.1 (n.s.)	1.3 (n.s.)	1.0 (n.s.)	0.4 (n.s.)	-1.0 (n.s.)
LX <sub>mixed</sub>	$\Delta_1$	-0.5 (n.s.)	-0.7 (n.s.)	-0.8 (n.s.)	-0.7 (n.s.)	0.2 (n.s.)	1.0 (n.s.)	1.1 (n.s.)	-0.9 (n.s.)
	$\Delta_2$	1.5 (n.s.)	0.8 (n.s.)	0.9 (n.s.)	0.6 (n.s.)	0.8 (n.s.)	2.2 (*)	2.5 (**)	1.4 (n.s.)

Significance codes: non-significant ( $p > 0.05$ ) 'n.s.';  $p \leq 0.05$  '\*';  $p < 0.01$  '\*\*';  $p < 0.001$  '\*\*\*'.

Abbreviations: Black Forest (BF), Lake Baldegg (LB), Upper Sûre Lake catchment-Luxembourg (LX).

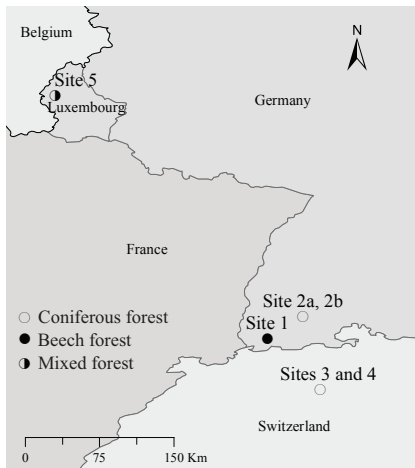


Figure 1: Map of all sampling sites.

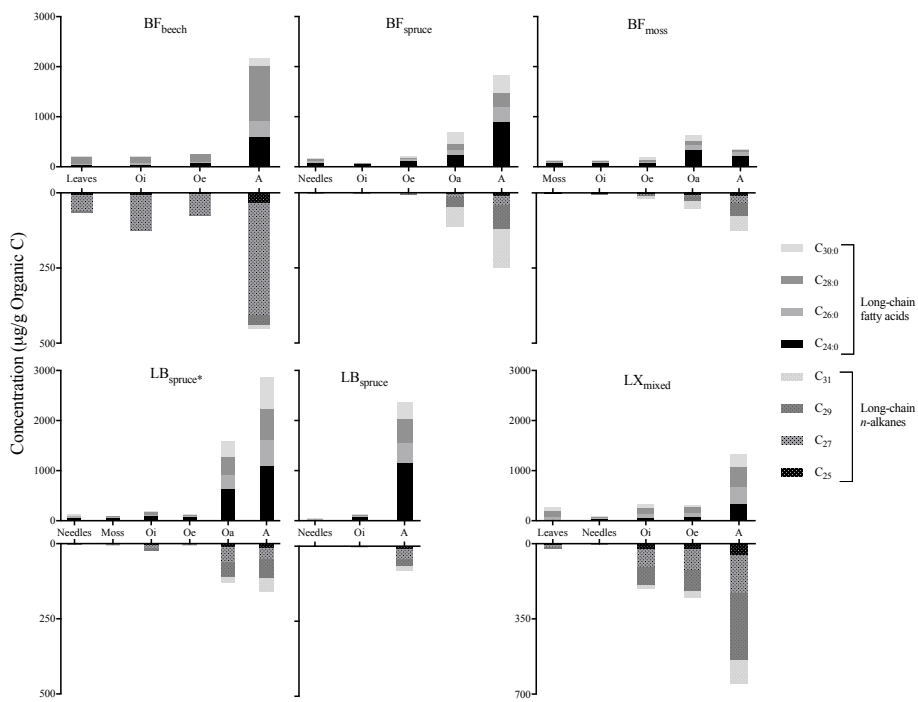


Figure 2: Concentration of long-chain fatty acids and *n*-alkanes in fresh plant biomass and underlying horizons from different sites (note the different scales of fatty acid and *n*-alkane concentration). Abbreviations: Black Forest (BF), Lake Baldegg (LB), Upper Sûre Lake catchment-Luxembourg (LX).

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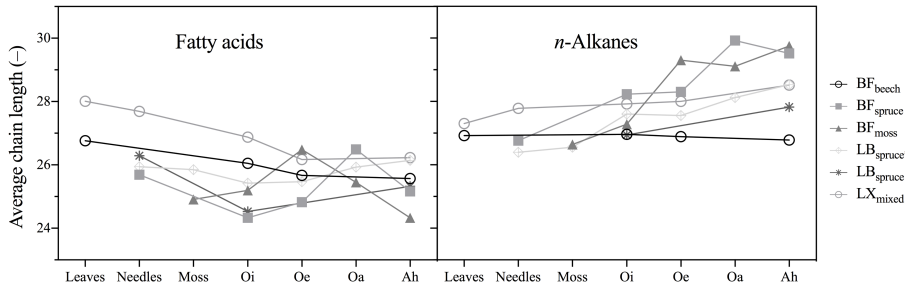
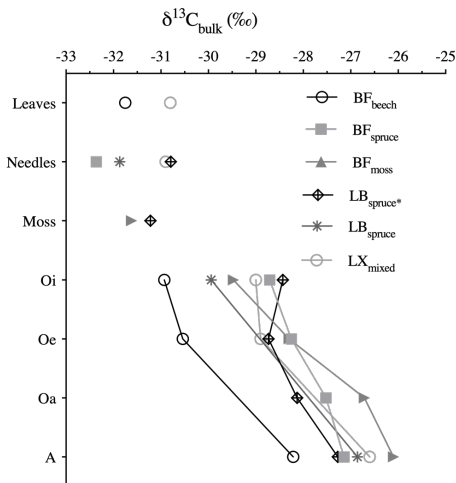


Figure 3: Average chain length (ACL) of fatty acids (ACL<sub>22-32</sub>) and *n*-alkanes (ACL<sub>21-33</sub>). Abbreviations: Black Forest (BF), Lake Baldegg (LB), Upper Sûre Lake catchment-Luxembourg (LX).



5 Figure 4: Bulk  $\delta^{13}\text{C}$  values (‰) of fresh plant biomass (leaves, needles & mosses) and underlying horizons. Abbreviations: Black Forest (BF), Lake Baldegg (LB), Upper Sûre Lake catchment-Luxembourg (LX).

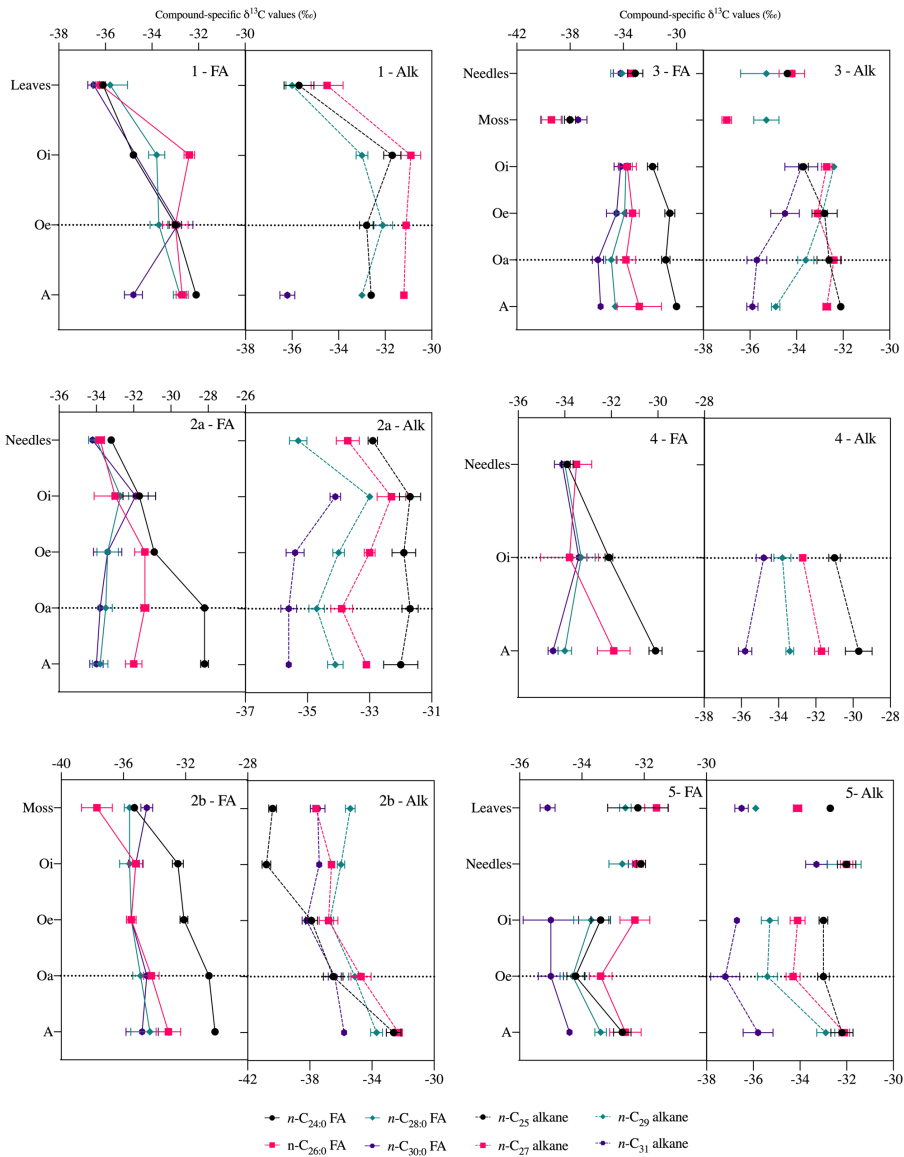


Figure 5: Compound-specific  $\delta^{13}\text{C}$  values of long-chain fatty acids ( $n\text{-C}_{24.0}$  to  $n\text{-C}_{30.0}$ ) and long-chain  $n$ -alkanes ( $n\text{-C}_{25}$  to  $n\text{-C}_{31}$ ) in fresh plant biomass (leaves, needles & mosses) and across the underlying horizons (mean  $\pm$  measurement error of 3 replicates). Black Forest (beech (1), spruce (2a), moss (2b)), Lake Baldegg (spruce\* (3), spruce (4)), Upper Sûre Lake catchment-Luxembourg (mixed forest (5)). Abbreviations: Fatty acids (FA),  $n$ -Alkanes (Alk).  $\delta^{13}\text{C}$  values generally remained stable after the horizon indicated by a horizontal dotted line.

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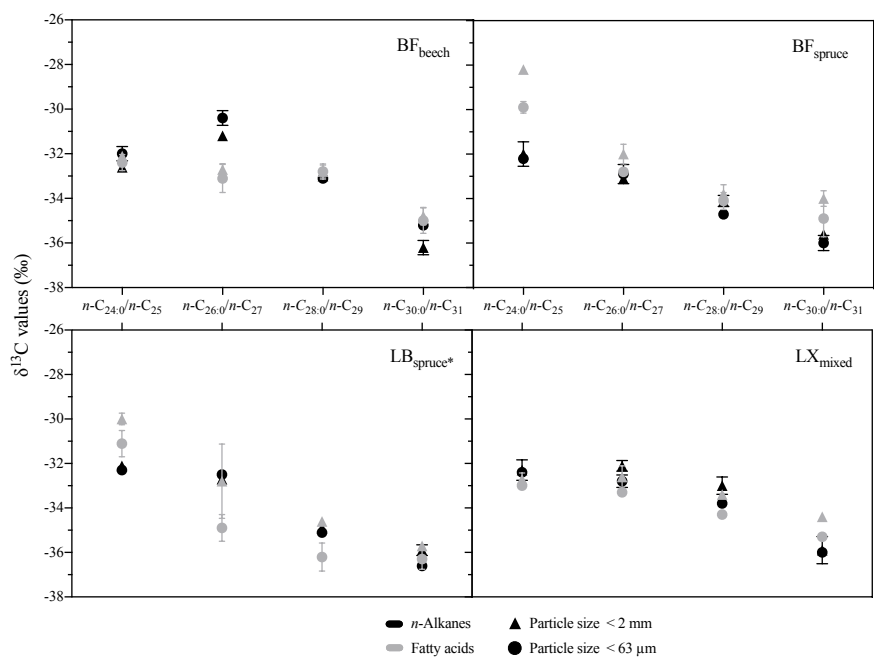


Figure 6: Comparison of the  $\delta^{13}\text{C}$  values from two particle-size fractions in soil from four sites (mean  $\pm$  measurement error of 3 replicates). Abbreviations: Black Forest (BF), Lake Baldegg (LB), Upper Sûre Lake catchment-Luxembourg (LX).