The fate of plant wax lipids in a model forest ecosystem under elevated CO₂ concentration and increased nitrogen deposition

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ABSTRACT

Atmospheric CO₂ concentration and nitrogen (N) deposition have been altered by anthropogenic activity and they affect global biogeochemical cycles. It is still not clear how these environmental changes influence the storage and cycling of organic matter (OM) in soils, although this plays a key role in the biogeochemistry of terrestrial ecosystems. Here, we used *n*-alkanes as biomarkers for plant-derived OM in specific soil fractions. We investigated the effect of elevated CO₂ concentration and increased N deposition on the molecular and isotopic ($\delta^{13}\text{C}$) composition of *n*-alkanes in above-ground and below-ground tree biomass (beech and spruce) and soil density fractions.

The *n*-alkane distribution patterns of spruce needles and root biomass were well reflected in those of particulate soil OM. Long chain (C₂₇₋₃₄) *n*-alkanes were preserved in soil by association with soil minerals, while mid-chain (C₂₀₋₂₇) *n*-alkanes from plant biomass and particulate soil OM were ultimately degraded due to lack of physical protection. Renewal of *n*-alkanes was lower in roots and spruce needles than in beech leaves. Similar low renewal of *n*-alkanes in soil reflected the low input of *n*-alkanes from plant biomass to the soil *n*-alkane pool. *n*-Alkane biosynthesis in beech leaves was modified under high N deposition and similar effects were observed for *n*-alkanes in mineral soil fractions and bulk soil. Here, different biosynthesis of *n*-alkanes in beech leaves under high N deposition consequently led to a modified input of *n*-alkanes from plant biomass to soil, with a relatively low

proportion of new (experimentally-derived) *n*-alkanes bound to soil minerals.

Keywords

terrestrial biogeochemistry, global environmental change, beech, spruce, roots, soil organic matter, soil density fractionation, biomarker, *n*-alkanes, compound-specific stable isotope analysis

1. Introduction

Human activity changes the environment globally through elevated atmospheric CO₂ concentration and increased atmospheric N deposition, which ultimately feed back to global biogeochemical cycles and the climate system (Ciais et al., 2013). Ecosystems that generally do not receive fertilization, like forests, are most affected by increased atmospheric N deposition, especially in industrialized regions, where N deposition is strongest (Galloway, 2014). This anthropogenic N fertilization affects plant growth and physiology, as well as soil organic matter (OM) stabilization and turnover (Reich and Hobbie, 2013; Fernández-Martínez et al., 2014). Although much is known about the effect of N on, e.g. tree growth and litter decomposition, the effect of N deposition on the soil C cycle in forests is still under debate (Janssens et al., 2010). Soils play a key role in the long term storage of C in terrestrial ecosystems (Stockmann et al., 2013), but the mechanisms behind the stabilization and destabilization of OM in soil remain unclear (Schmidt et al., 2011; Cotrufo et al., 2015; Doetterl et al., 2015).

Plant wax lipids can be used as biomarkers for tracing plant-derived OM in soil (Kögel-Knabner, 2002; Amelung et al., 2008). *n*-Alkanes are components of plant wax and their distribution patterns and C isotopic composition ($\delta^{13}\text{C}$) vary between different plant species (Diefendorf et al., 2011, 2015; Bush and McInerney, 2013) and between above-ground vs. below-ground plant biomass (Huang et al., 2011). This information has been

used for source apportionment of OM in soils of agricultural (Lichtfouse et al., 1994; Wiesenberg et al., 2004), grassland (Wiesenberg et al., 2008a) and forest ecosystems (Marseille et al., 1999; Quna et al., 2006). However, only a few studies have used isotopic labeling in combination with compound-specific stable isotope analysis in forests (Feng et al., 2010) since, in contrast to agricultural and grassland ecosystems, introduction of a ^{13}C label into forest ecosystems cannot be achieved via vegetation change. Although some experiments applied fumigation of forest ecosystems with ^{13}C -depleted CO_2 (Norby and Zak, 2011; Bader et al., 2013), only few studies have made use of a ^{13}C label to determine the isotopic composition of plant wax compounds in tree biomass and determine their input to soil OM (Feng et al., 2010; Griepentrog et al., 2015). Furthermore, most studies investigated bulk soil (Nierop, 1998; Marseille et al., 1999; Bull et al., 2000) and, although studies of soil particle size fractions are available (Cayet and Lichtfouse, 2001; Quna et al., 2006), investigations of *n*-alkanes in soil density fractions are scarce (Wiesenberg et al., 2010; Hfle et al., 2013) and to our knowledge not available for forest ecosystems. It is therefore not clear how *n*-alkane (isotope) patterns in above-ground and below-ground tree biomass are linked to the corresponding *n*-alkane patterns in soil density fractions of forest soils.

In this study we investigated model forest ecosystems in open-top chambers exposed to ambient and elevated (^{13}C -depleted) CO_2 concentration in combination with two levels of artificial atmospheric N deposition for 4

years. We analyzed the molecular and isotope ($\delta^{13}\text{C}$) composition of *n*-alkanes in above-ground and below-ground tree biomass of beech and spruce, as well as in bulk soil and soil density fractions. The target was to study how *n*-alkane distribution patterns and isotope composition in plant biomass are linked with those in specific soil fractions. We tested if elevated CO_2 concentration and/or increased N deposition affect the molecular and isotope composition of *n*-alkanes in above-ground and below-ground tree biomass and soil density fractions. Furthermore, we used the different isotope signatures of ambient and ^{13}C -depleted CO_2 to trace the *n*-alkanes in different plant and soil compartments and to determine the amount of isotope label taken up during the experimental period, which is theoretically linked to the turnover of *n*-alkanes in these ecosystem compartments.

2. Material and methods

2.1. *Experimental setup*

Soil and plant samples from a free air CO₂ enrichment (FACE) experiment were used. The experiment was conducted during four growing seasons from 1994 to 1998. The detailed experimental setup is given by Egli et al. (1998). In brief, forest ecosystems were established in open-top chambers (3 m high, 3 m diameter) with a mixture of beech (*Fagus sylvatica*) and spruce (*Picea abies*) trees planted on an acidic soil with sandy loamy texture (Haplic Alisol, FAO 1990). At the beginning of the experiment the beech trees were 2-3 yr old, while the spruce trees were 4 yr old and there was no forest floor on the soils.

The model ecosystems were treated for 4 yr with two values of atmospheric CO₂ concentration (370 and 570 $\mu\text{mol/mol}$) in combination with two values of artificial atmospheric N deposition (7 and 70 kg NH₄NO₃-N/ha/yr). N deposition was applied with irrigation water twice a week through nozzles above the plant canopy and a transparent roof automatically closed at the onset of rain to prevent external water and nutrient input from rain. These ecosystems were isotopically labeled continuously via the atmosphere, since the carbon isotope signature ($\delta^{13}\text{C}$) of the elevated CO₂ treatment was 16‰ lower than the treatment with ambient CO₂ (Hagedorn et al., 2001). The following four treatment combinations were investigated: ambient CO₂ and low N; elevated CO₂ and

low N; ambient CO₂ and high N; elevated CO₂ and high N. Each of the 4 treatment combinations was applied in triplicate in the field.

After 4yr of treatment, soil samples were taken from 0-10 cm depth of mineral soil horizons (without organic layer). Trees were harvested and separated into above-ground and below-ground biomass (beech leaves and roots, spruce needles and roots, respectively). Soil and plant samples were dried (60 °C) immediately after sampling and stored in sealed containers in a fully air conditioned archive room (17 °C) with low air humidity (< 40%) until analysis.

2.2. Soil density fractionation

Bulk soil samples were fractionated according to the concept of Golchin et al. (1994). In brief, bulk soil was separated into a free light fraction (fLF), occluded light fraction (oLF) and total heavy fraction (tHF) using a combination of density fractionation and ultrasonic dispersion. Selection of density and ultrasonic dispersion energy was based on pre-experiments to maximize the organic C content of light fractions (Cerli et al., 2012; Griepentrog and Schmidt, 2013).

The soil density fractionation aimed at separating distinct soil fractions that conceptually related to different stabilization mechanisms of OM in soil, including (e.g. Dorodnikov et al., 2011): OM not physically protected from degradation (fLF), OM protected against degradation by way of

occlusion in soil aggregates (oLF) and OM protected against degradation by association with soil minerals (tHF).

A detailed description of the fractionation procedure is given by Griepentrog et al. (2014). In brief, sodium polytungstate (SPT 0; Tungsten Compounds, Grub am Forst, Germany) solution with a density of 1.6 g/cm³ was used for density fractionation and already used SPT solutions were collected and recycled according to Six et al. (1999). Bulk soil samples (dried and sieved < 2 mm) were suspended in SPT solution and centrifuged. The floating material (fLF) was collected, filtered and rinsed with de-ionized water to remove residual SPT. The remaining material was resuspended in SPT and ultrasonically dispersed. The ultrasonic equipment was calorimetrically calibrated according to Schmidt et al. (1999). After centrifugation, the floating material (oLF) was collected, filtered and rinsed with deionized water. The remaining soil material (tHF) was rinsed 3 x with deionized water followed by centrifugation each time. All fractions were freeze-dried and milled with a ball mill prior to analysis. All soil samples were fractionated in triplicate and pooled for further analysis. On average we recovered > 99% of the initial sample mass (Griepentrog et al., 2014).

2.3. n -Alkane (isotope) analysis

Plant biomass and soil fractions were extracted with a mixture of dichloromethane (DCM)/MeOH (9/1; v/v) using microwave extraction for 1 h at 1600 W (MARS XpressTM Microwave Reaction System; CEM Corporation,

Matthews, NC, USA). Solid phase extraction with silica gel and 5% KOH (Silica 60 + 5% KOH, 63-200 μm , Margot Köhnen-Willsch, Jülich, Germany) was used to separate each extract into neutral and acidic lipids (Wiesenberg and Gocke, 2016). First, neutral lipids were eluted with DCM and acidic lipids with DCM/HCO₂H (99/1; v/v). Solid phase extraction with activated silica gel (63-200 μm , 120 °C) was used to further separate the neutral fraction. The aliphatic hydrocarbon fraction containing *n*-alkanes was eluted with hexane. The fraction was reduced in volume, transferred to a vial and stored dry until analysis (Wiesenberg and Gocke, 2016).

Assignment and relative abundance of individual *n*-alkanes were determined using a gas chromatograph with PTV injector (Trace GC, Thermo Scientific, Bremen, Germany) coupled to a quadrupole mass spectrometer (Trace DSQ, Thermo Scientific, Bremen, Germany). The gas chromatography (GC) column (Agilent VF-5ms, 30 m x 250 μm i.d., 0.25 μm film thickness) temperature program was: 50 °C (1 min) to 250 °C at 25 °C/min, then to 350 °C (held 5 min) at 10 °C/min. The carrier gas was He at 1 ml/min.

Stable carbon isotope values ($\delta^{13}\text{C}$) of individual *n*-alkanes were determined using a Thermo Trace GC Ultra with PTV injector connected to a Thermo Delta Plus XP via a Thermo GC/C III interface (Thermo Scientific, Bremen, Germany). The GC column (Agilent VF-5ms, 30 m x 250 μm i.d., 0.25 μm film thickness) temperature program was: 50 °C (2 min) to 180 °C

at 50 °C/min, then to 340 °C (held 6 min) at 5 °C/min. The carrier gas was He at 1 ml/min.

¹³C abundance is expressed relative to the Vienna Pee Dee Belemnite (VPDB) reference standard in per mill (δ¹³C‰). Calibration against the VPDB scale was performed using the A5 hexane reference (C₁₆ – C₃₀; A. Schimmelmann, Indiana University, Bloomington, Indiana, USA) traceable to the primary standards NBS 19 and L-SVEC. Reproducibility and stability of δ¹³C values were evaluated with pulses of CO₂ reference gas and a set of references was analyzed in between every 4 samples. Standard deviation on a reference injection was 0.3‰ on average and uncertainty on the VPDB scale was 0.1‰ (average for all measured *n*-alkanes).

2.4. Calculations

Average chain length (ACL) is the weighted average of a selected range of *n*-alkanes with different chain lengths and was calculated according to the following equation (e.g. Killops and Killops, 2005):

$$ACL = \sum(n \cdot C_n) / \sum(C_n)$$

where *n* is the number of carbons for total *n*-alkanes (C₂₀₋₃₄) and *C_n* the relative abundance of each *n*-alkane with *n* carbons in the respective carbon number range.

Carbon preference index (CPI) is a measure for the predominance of odd/even numbered n -alkanes and was calculated for total n -alkanes (C₂₀₋₃₄) according to the following equation (e.g. Killops and Killops, 2005):

$$CPI = \left[2 \cdot \sum_{odd} (C_{21-33}) \right] / \left[\sum_{even} (C_{20-32}) + \sum_{even} (C_{22-34}) \right]$$

Isotope values ($\delta^{13}C$) for mid-chain (C₂₀₋₂₇) and long chain (C₂₇₋₃₄) n -alkanes were separately calculated as weighted means of the isotope values of the individual n -alkanes (e.g. Wiesenberg et al., 2008b):

$$\bar{\delta} = \sum_{i=a}^b w_i \cdot \delta_i$$

where a and b represent the lower and upper limits of the respective carbon number range, w_i the relative abundance of the individual n -alkane and δ_i the isotope value of the individual n -alkane.

2.5. Statistical analysis

In general, the mean is given along with the standard error for replicate measurements (Webster, 2001). Means and standard errors are based on three field replicates. However, field replicate samples of plant biomass needed to be pooled for lipid extraction, due to limited sample amount. Here, the means and standard errors are derived from replicated laboratory

measurements of the available fractions. Two-way analysis of variance was used to test the statistical significance of treatment effects (CO₂ concentration, N deposition) and their interaction (Webster, 2007) on relative abundance, ACL and CPI of *n*-alkanes in plant biomass and soil fractions.

3. Results

3.1. n-Alkane distribution patterns

Overall, a wide range of *n*-alkanes was detected ($C_{20} - C_{34}$), and showed a maximum at C_{27} for all types of samples (above-ground and below-ground plant biomass, soil fractions and bulk soil) except for spruce roots, where C_{25} had the highest relative abundance (Fig. 1; Supplementary Table 1). In plant biomass, beech leaves consisted mainly of C_{27} and C_{29} ($82 \pm 1\%$ and $9 \pm 1\%$, respectively). In contrast, spruce needles and root biomass of both plant species showed no dominance of single *n*-alkanes and a higher relative abundance of mid-chain *n*-alkanes ($\leq C_{26}$), with a greater contribution of even *n*-alkanes (Fig. 1; Supplementary Table 1). Similar to spruce needles and root biomass, both light fractions (free LF and occluded LF) were characterized by a high relative abundance of mid-chain *n*-alkanes ($\leq C_{26}$), with a high proportion of even *n*-alkanes (Fig. 2; Supplementary Table 1). In contrast, bulk soil and total heavy fraction contained a high relative abundance of long chain *n*-alkanes ($\geq C_{27}$) with a high proportion of odd *n*-alkanes (Fig. 2; Supplementary Table 1). Treatment with elevated CO_2 concentration as well as increased N deposition did not significantly ($p > 0.05$) affect distribution patterns of *n*-alkanes in plant biomass, soil fractions and bulk soil.

3.2. n-Alkane molecular proxies

The distribution patterns of *n*-alkanes in plant biomass (Fig. 1; Supplementary Table 1) and soil fractions (Fig. 2; Supplementary Table 1) are reflected in average chain length (ACL) and carbon preference index (CPI) values (Fig. 3; Supplementary Table 2). Light fractions, root biomass and spruce needles were characterized by $ACL < 27$ and low CPI ranging from 1.0 ± 0.0 to 1.5 ± 0.2 (Fig. 3; Supplementary Table 2). In contrast, bulk soil and total heavy fractions showed $ACL > 27$ and higher CPI of 3.1 ± 0.3 and 3.0 ± 0.2 , respectively (Fig. 3; Supplementary Table 2). Beech leaves had highest CPI of 17.9 ± 0.6 , since they consisted almost exclusively of C_{27} and C_{29} (Fig. 1; Supplementary Table 1). Treatment with elevated CO_2 concentration as well as increased N deposition did not significantly ($p > 0.05$) affect ACL or CPI values in plant biomass, soil fractions and bulk soil.

3.3. n-Alkane carbon isotope composition

Based on the distribution patterns, *n*-alkanes were grouped into mid-chain (C_{20-27}) and long chain (C_{27-34}) *n*-alkanes and weighted means of $\delta^{13}C$ values were calculated separately for both groups (Fig. 4; Supplementary Tables 3 and 4). We included the C_{27} *n*-alkane in both groups due to the unimodal distributions and the fact that it was the most abundant in all the samples (Figs. 1 and 2; Supplementary Table 1) and therefore to allow more robust weighted mean values. Weighted mean values of $\delta^{13}C$ values ranged from $-27.0 \pm 0.1\text{‰}$ to $-30.6 \pm 0.1\text{‰}$ under ambient CO_2 and from $-27.3 \pm 0.2\text{‰}$ to -

40.8 $\pm$ 0.3‰ under elevated (^{13}C depleted) CO_2 (Fig. 4; Supplementary Tables 3 and 4).

In plant biomass, no differences in $\delta^{13}\text{C}$ between mid-chain and long chain n -alkanes were observed, except for spruce needles that showed higher values for mid-chain than long chain n -alkanes (from $0.3 \pm 0.1\text{‰}$ to $2.3 \pm 0.1\text{‰}$). In all soil density fractions and bulk soil, mid-chain n -alkanes were generally more enriched in ^{13}C vs. long chain n -alkanes (from $0.6 \pm 0.1\text{‰}$ to $4.3 \pm 0.6\text{‰}$).

In plant biomass, beech leaves were more depleted in ^{13}C than spruce needles and roots of both species under ambient CO_2 concentration (Fig. 4; Supplementary Table 3). Under elevated (^{13}C -depleted) CO_2 concentration, above-ground biomass was more depleted in ^{13}C than below-ground biomass. For soil fractions, both light fractions were enriched in ^{13}C vs. the heavy fraction and bulk soil under ambient CO_2 concentration (Fig. 4; Supplementary Table 4). Under elevated (^{13}C -depleted) CO_2 concentration, similar patterns were observed except for long chain n -alkanes in the free light fraction (from $-31.2 \pm 0.3\text{‰}$ to $-33.1 \pm 0.6\text{‰}$), which were more depleted than in root biomass (Fig. 4; Supplementary Tables 3 and 4). N deposition affected $\delta^{13}\text{C}$ values of n -alkanes in above-ground and below-ground plant biomass as well as soil fractions and bulk soil (Fig. 4, Supplementary Tables 3 and 4). In many cases, higher $\delta^{13}\text{C}$ values were observed for high vs. low N deposition (Fig. 4; Supplementary Tables 3 and 4).

Differences in $\delta^{13}\text{C}$ values between ambient and ^{13}C -depleted CO_2 treatment were greatest for beech leaves (from $9.4 \pm 0.2\text{‰}$ to $12.1 \pm 0.3\text{‰}$), followed by spruce needles ($1.7 \pm 0.2\text{‰}$ to $4.0 \pm 0.1\text{‰}$) and lowest for root biomass ($-0.2 \pm 0.2\text{‰}$ to $1.8 \pm 1.5\text{‰}$) of both plant species (Fig. 5, Supplementary Table 3). In soil fractions and bulk soil, differences between $\delta^{13}\text{C}$ values of ambient and ^{13}C -depleted CO_2 treatment were generally low ($< 1\text{‰}$, Fig. 5; Supplementary Table 4). Exceptions were long chain n -alkanes (C_{27-34}) in free light fractions under both high and low N deposition ($2.3 \pm 0.3\text{‰}$ and $3.1 \pm 0.6\text{‰}$, respectively), and for the heavy fraction ($1.6 \pm 0.2\text{‰}$) and bulk soil ($1.0 \pm 0.2\text{‰}$) under low N deposition (Fig. 5; Supplementary Table 4). In many cases, N deposition affected the differences in $\delta^{13}\text{C}$ values between ambient and ^{13}C -depleted CO_2 treatment for mid-chain and long chain n -alkanes in beech leaves and for long chain n -alkanes in heavy fraction and bulk soil, with lower values under high vs. low N deposition (Fig. 5; Supplementary Tables 3 and 4).

4. Discussion

4.1. n-Alkane patterns in tree tissue and forest soil fractions

Distribution patterns of beech leaves with C₂₇ as the dominant *n*-alkane (Fig. 1) are generally observed (e.g. Gülz et al., 1992). Spruce needles, root biomass (Fig. 1) and light soil fractions (Fig. 2) showed higher relative amounts of mid-chain *n*-alkanes and comparatively low CPI values. The origin of mid-chain *n*-alkanes with low CPI values is, however, not clearly constrained in the literature. First, mid-chain *n*-alkanes with low CPI values can have a non-biological source, such as contamination with fossil fuel (combustion) products (Lichtfouse and Eglinton, 1995) or combustion induced thermal breakdown of long-chain *n*-alkanes (Wiesenberg et al., 2009), which could be ruled out here. Moreover, Jansen et al. (2006) found leaves and roots of several species from the Ecuadorian Andes to contain mid-chain *n*-alkanes with low CPI values and Kuhn et al. (2010) found that such *n*-alkanes also dominated in Australian woodland soils under Acacia trees. Therefore, the low CPI values of mid-chain *n*-alkanes in soil can represent a biological (non-diagenetic) signature of the source plant biomass (Kuhn et al., 2010). This seems to be valid in our case, due to the close resemblance of the distribution patterns between roots and particulate soil OM (Fig. 3). Microorganisms are another potential source of mid-chain *n*-alkanes in roots (Nguyen Tu et al., 2000). It is therefore also possible that the mid-chain *n*-alkanes in roots and light soil fractions are derived from

microorganisms (especially fungi) living in close connection with roots (mycorrhiza) and in particulate soil OM (Jones et al., 2009).

It has been shown that the *n*-alkane content of conifer needles and roots is several times lower than of leaves (Jansen et al., 2006; Diefendorf et al., 2011). Nevertheless, the distribution patterns of *n*-alkanes in needles and roots are well reflected in that of both light fractions (Fig. 3), showing that these compounds contribute considerably to particulate soil OM. Although it has been assumed that soil OM is derived mainly from above-ground plant input, it is increasingly being recognized that OM input from roots often accounts for a large proportion of soil OM (e.g. Mambelli et al., 2011; Xia et al., 2015). Huang et al. (2011) showed that roots are an important source of lipid biomarkers in peatlands and in some cases contribute more *n*-alkanes than their above-ground counterparts. The contribution of root-derived *n*-alkanes might also be important for our acidic soils, where degradation of OM is often limited (van Bergen et al., 1998).

In contrast to roots and light fractions, which exhibit a relatively high amount of mid-chain *n*-alkanes with low CPI values, we found that heavy (mineral) soil fractions and bulk soil showed a comparatively low amount of mid-chain *n*-alkanes and a predominance of odd long-chain *n*-alkanes (Figs. 2, 3). Similar patterns were observed by Marseille et al. (1999) for spruce needles and the corresponding organic soil layer and mineral soil. Also, Höfle et al. (2013) found similar patterns in light soil fractions and bulk soils from the arctic tundra, but unfortunately they did not analyze plant

biomass. Furthermore, Quénée et al. (2006) showed that long chain n -alkanes are found mainly in the fine mineral soil fractions and a high abundance of mid-chain n -alkanes with low CPI values in root biomass. The distribution patterns in heavy fractions closely resemble those of bulk soil in our study (Figs. 2, 3). This is not surprising, since the heavy fraction OM contributes 71% to the total OM of bulk soil (Griepentrog et al., 2014).

The fact that mid-chain n -alkanes show minor proportions in bulk soil and heavy fractions might be attributed to (i) generally lower n -alkane concentration in needles and roots (which have a relatively high amount of mid-chain n -alkanes) compared with leaves (which have almost no mid-chain n -alkanes; Diefendorf et al., 2011) and therefore (ii) mid-chain n -alkanes from needles and roots are diluted by a large amount of long-chain n -alkanes from beech leaves. Furthermore, n -alkane concentrations are not only lower in the below-ground vs. above-ground plant biomass, but also in light vs. heavy fractions (Wiesenberg et al., 2010). Here, selective preservation of long chain n -alkanes has also been proposed (Lichtfouse et al., 1995, 1998) and can be attributed to different stabilization processes in soil (von Lützow et al., 2006). Particulate soil OM is not as effectively protected from decomposition as mineral-associated soil OM (von Lützow et al., 2006). Since n -alkanes are not primarily produced in-situ in mineral soil fractions, they have to be transferred to soil minerals from plant, microbial and/or particulate soil OM. It is therefore possible that plant biomass and particulate soil OM, which contain a relatively large amounts of mid-chain

n-alkanes, are ultimately degraded during initial decomposition, due to the lack of physical protection. By contrast, long chain *n*-alkanes are preserved in soil over the long term due to protection from decomposition via mineral association of OM (Kleber et al., 2015). Similar results have been obtained for fatty acids (FAs) within this experiment that showed higher ratio values of long chain FAs (C₂₀₋₃₀) to short chain FAs (C₁₆₊₁₈) in mineral soil fractions vs. plant biomass and particulate soil OM, also suggesting that long chain FAs are preferentially stabilized via association with soil minerals (Griepentrog et al., 2015).

*4.2. Isotopic composition ($\delta^{13}\text{C}$) of *n*-alkanes*

The range of $\delta^{13}\text{C}$ values under ambient CO₂ (from -27.0 to -30.6‰) is in the range generally measured for terrestrial C₃ plants (Chikaraishi, 2014) and those for the beech leaves are well in line with Lockheart et al. (1997).

Higher $\delta^{13}\text{C}$ values for mid-chain vs. long chain *n*-alkanes were observed for spruce needles as well as soil density fractions and bulk soil (Fig. 4). Also Nguyen Tu et al. (2004) showed that mid-chain *n*-alkanes (especially even ones) were enriched in ¹³C vs. long chain ones in *Ginkgo biloba* leaves. Processes leading to these isotope variations between *n*-alkanes in plant biomass are unclear, but a possible reason could be that different homologues can be produced at various stages of the growing season, which in turn has an effect due to changes in environmental conditions and plant physiology (Nguyen Tu et al., 2004). Furthermore, even *n*-alkanes could be

degradation products of other alkyl lipids and hence be heavier (Dinel et al., 1990; Amblès et al., 1994). It is, however, difficult to determine the extent of degradation, since degradation products are indistinguishable from their biological precursors (Otto and Simpson, 2005).

In soil, mid-chain *n*-alkanes might be sourced in part from soil microorganisms, which are generally enriched in ^{13}C vs. plant biomass (Bowling et al., 2008). Little is known about microorganism-derived *n*-alkanes, but mid-chain *n*-alkanes have been found in fungi (Fisher et al., 1978). *n*-Alkanes in both light fractions were enriched in ^{13}C compared with heavy fractions and bulk soils (Fig. 4), in line with other studies that analyzed $\delta^{13}\text{C}$ values of *n*-alkanes in particle size fractions (Cayet and Lichtfouse, 2001; Quénéa et al., 2006). The ^{13}C enrichment in light fractions might be due to microorganisms (especially fungi) that are associated with the rhizosphere and particulate OM fractions in our soil (Wiemken et al., 2001a,b). Higher relative amounts of fungal residues (amino sugars) were actually found in light vs. heavy soil fractions within our experiment (Griepentrog et al., 2014). Also the fact that mid-chain *n*-alkanes in root biomass were enriched in ^{13}C vs. beech leaves under ambient CO_2 (Fig. 4) points towards a possible contribution from microorganisms that are generally associated with root biomass (Jones et al., 2009). Similar results from the same experiment were found for short chain FAs (C_{16+18}) that generally showed higher $\delta^{13}\text{C}$ values for below-ground than above-ground

plant biomass and further support a potential microbial contribution to below-ground lipids (Griepentrog et al., 2015).

N deposition frequently affected $\delta^{13}\text{C}$ values of *n*-alkanes and in most cases higher $\delta^{13}\text{C}$ values were observed for high vs. low N deposition (Fig. 4). The results are well in line with FA data from the same experiment (Griepentrog et al., 2015) and with another study of the isotopic composition of *n*-alkanes in *Betula pendula* seedlings under N deposition (Huang et al., 1999). A possible reason for the ^{13}C enrichment under high N deposition could be decreased isotope fractionation during photosynthesis due to changes in plant physiology (Huang et al., 1999). It could be shown that increased N deposition leads to narrower stomatal pores and to a decrease in the density of stomata (Frey et al., 1996). Concurrently, N deposition increases plant growth and stimulates photosynthesis, which consequently leads to a reduction in ^{13}C discrimination during photosynthesis (Farquhar et al., 1989). Furthermore, narrower stomatal pores and decreased stomata density under high N deposition result in less water transpiration by plants, which in turn theoretically leads to reduced production of wax components or a reduction in their renewal rate to regulate water diffusion via the cuticula (Shepherd and Griffiths, 2006). Lower renewal rate of *n*-alkanes results in their greater persistence and in accumulation of ^{13}C -enriched *n*-alkanes in cuticular plant wax. A similar N deposition effect in soil fractions can be attributed to the observed changes in $\delta^{13}\text{C}$ values of *n*-alkanes from plant biomass input.

4.3. Experimentally-derived plant waxes

Differences between *n*-alkane $\delta^{13}\text{C}$ values of ambient and ^{13}C -depleted CO_2 treatment reflect the incorporation of the isotope label during the time span of the experiment (4 yr) and therefore the biosynthesis of new experiment-derived *n*-alkanes in plant tissue. Within different soil fractions, it is a measure of the incorporation and turnover of *n*-alkanes therein (assuming a steady state of *n*-alkane concentration). Differences in *n*-alkane $\delta^{13}\text{C}$ values between ambient and ^{13}C -depleted CO_2 treatment (and thus *n*-alkane turnover) were highest for beech leaves, followed by spruce needles, and root biomass of both plant species were lowest (Fig. 5). This suggests not only that the amount of *n*-alkanes is lower in coniferous leaves and root biomass than in deciduous leaves (Diefendorf et al., 2011), but also that the biosynthesis of *n*-alkanes is different in roots and coniferous biomass. The lower *n*-alkane turnover in roots than above-ground plant biomass is in contrast to FAs in the same experiment (Griepentrog et al., 2015). In the latter, differences between above-ground and below-ground biomass were relatively small, suggesting that FAs are renewed at a higher rate in roots than *n*-alkanes. In general, differences between $\delta^{13}\text{C}$ values of ambient and ^{13}C -depleted CO_2 in soil fractions and bulk soil were lower than in above-ground plant biomass (Fig. 5), reflecting a relatively low input of new (experiment-derived) *n*-alkanes from plant biomass to the soil *n*-alkane pool after the 4 yr of the experiment. Similar results were shown for agricultural

(Cayet and Lichtfouse, 2001; Wiesenberg et al., 2004) and grassland (Wiesenberg et al., 2008b) ecosystems.

N deposition affected the differences in $\delta^{13}\text{C}$ values between ambient and ^{13}C -depleted CO_2 treatment for mid-chain and long chain *n*-alkanes in beech leaves, with lower values under high vs. low N deposition (Fig. 5). This suggests that the biosynthesis of *n*-alkanes in beech leaves is modified under high N deposition compared with low N deposition. Interestingly, *n*-alkane distributions were not affected by increased N deposition in this experiment. This shows that plants are able to regulate the biosynthesis rate of *n*-alkanes in the plant cuticles, while concurrently the mechanism of *n*-alkane biosynthesis (chain length) remains constant. Similar results were obtained for FAs in this experiment, showing that N deposition affects FA turnover but not FA distributions (Griepentrog et al., 2015). The results are in contrast to the frequently discussed process whereby plants react to environmental stress with increased production of lipids with different chain length (Shepherd and Griffiths, 2006).

Long chain *n*-alkanes in mineral soil fractions and bulk soil also showed smaller differences between $\delta^{13}\text{C}$ values of ambient and ^{13}C -depleted CO_2 under high vs. low N deposition (Fig. 5). Here, the altered biosynthesis of *n*-alkanes in beech leaves under high N deposition possibly leads to reduced input of *n*-alkanes from plant biomass to the soil and consequently to a relatively low proportion of new (experiment-derived) *n*-alkanes bound to soil minerals. Another possible reason is retarded decomposition of old (pre-

experiment) OM under high N deposition, which can be explained by reduced mining of OM for N by microorganisms if inorganic N is available (Fontaine et al., 2011). This process appears to be particularly important in mineral soil fractions where OM is protected from microbial degradation due to association with soil minerals. The results here are in good agreement with data from the same experiment that also found retarded decomposition of old (pre-experiment) OM under high N deposition for total organic carbon (Hagedorn et al., 2003), microbial amino sugars (Griepentrog et al., 2014) and FAs (Griepentrog et al., 2015), suggesting that this mechanism is of general relevance as it is consistent for a variety of plant and microbial biomarkers.

5. Conclusions

Spruce needles, roots of both tree species and light soil density fractions contained relatively high amounts of mid-chain *n*-alkanes with comparatively low predominance of odd-numbered *n*-alkanes. The distribution patterns in spruce needles and roots were well reflected in those of light soil density fractions, showing that these compounds make up a considerable proportion of particulate OM in soil. Heavy soil density fractions and bulk soil showed a comparatively low amount of mid-chain *n*-alkanes and a predominance of odd long chain *n*-alkanes, suggesting that mid-chain *n*-alkanes of plant biomass and particulate soil OM are ultimately degraded due to a lack of physical protection, while long chain *n*-alkanes are better preserved in soil via mineral association of OM.

Incorporation of isotope label into *n*-alkanes was greatest for beech leaves and relatively lower for spruce needles and root biomass of both plant species, suggesting that the renewal rate of *n*-alkanes was comparatively low in roots and coniferous biomass. Incorporation of isotope label into soil *n*-alkanes generally reflected a relatively low input of new (experiment-derived) *n*-alkanes from plant biomass to the soil *n*-alkane pool after the four years of the experiment.

N deposition affected the incorporation of isotope label into *n*-alkanes of beech leaves, suggesting that the biosynthesis of *n*-alkanes is modified under high N deposition compared with low N deposition. A similar N effect on the incorporation of isotope label was observed for *n*-alkanes in mineral

soil fractions and bulk soil. Here, the altered biosynthesis of *n*-alkanes in beech leaves under high N deposition leads to reduced input of *n*-alkanes from plant biomass to the soil and consequently to a relatively low proportion of new (experiment-derived) *n*-alkanes bound to soil minerals. Furthermore, these effects can be interpreted as a retarded decomposition of old (pre-experiment) OM under high N deposition, which can be explained by reduced N mining of OM by microorganisms if inorganic N is available. This process was already consistently found for a variety of plant and microbial biomarkers within this experiment and appears to be particularly important in mineral soil fractions, where OM is effectively protected from microbial degradation by association with soil minerals.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at ...

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Figure captions

Fig. 1. Distributions (relative abundance) of *n*-alkanes in above-ground (beech leaves and spruce needles) and below-ground (beech and spruce roots) tree biomass. Treatment with elevated CO₂ concentration as well as increased N deposition did not significantly ($p > 0.05$) affect distribution patterns of *n*-alkanes in plant biomass and therefore averages of all four treatments are shown.

Fig. 2. Distributions (relative abundance) of *n*-alkanes in soil density fractions (free light fraction (LF), occluded light fraction (LF), total heavy fraction (HF)) and bulk soil. Treatment with elevated CO₂ concentration as well as increased N deposition did not significantly ($p > 0.05$) affect distribution patterns of *n*-alkanes in soil density fractions and bulk soil and therefore averages of all four treatments are shown.

Fig. 3. Average chain length (ACL, C₂₀₋₃₄) and carbon preference index (CPI, C₂₀₋₃₄) of *n*-alkanes in above-ground (beech leaves and spruce needles) and belowground (beech and spruce roots) tree biomass as well as soil density fractions (free light fraction (LF), occluded light fraction (LF), total heavy fraction (HF)) and bulk soil. Treatment with elevated CO₂ concentration as well as increased N deposition did not significantly ($p > 0.05$) affect ACL or CPI values of *n*-alkanes in tree biomass, soil density fractions and bulk soil and therefore averages of all four treatments are shown.

Fig. 4.

$\delta^{13}\text{C}$ values of (a) mid-chain C_{20-27} and (b) long chain C_{27-34} *n*-alkanes in above-ground (beech leaves, spruce needles) and below-ground (beech roots, spruce roots) plant biomass, soil density fractions (free light fraction (LF), occluded light fraction (LF), total heavy fraction (HF)) and bulk soil. Ambient and ^{13}C -depleted CO_2 concentration were applied in combination with low and high nitrogen (N) deposition for 4 yr. Note that the isotopic difference between ambient and ^{13}C -depleted CO_2 was ca. 16‰.

Fig. 5. Difference in $\delta^{13}\text{C}$ values of *n*-alkanes between ambient and ^{13}C -depleted CO_2 treatment after 4 yr for (a) mid-chain C_{20-27} and (b) long chain C_{27-34} *n*-alkanes in above-ground (beech leaves, spruce needles) and below-ground (beech roots, spruce roots) plant biomass, soil density fractions (free light fraction (LF), occluded light fraction (LF), total heavy fraction (HF)) and bulk soil. Note that the isotopic difference between ambient and ^{13}C -depleted CO_2 was ca. 16‰.

Fig. 1.

Fig. 2 .

Fig. 3 .

Fig. 4 .

Fig. 5 .

Supplementary table captions

Supplementary Table 1

Relative abundance (i%) of *n*-alkanes (C₂₀₋₃₄) in plant biomass (beech leaves, spruce needles, beech roots, spruce roots), soil density fractions (free light fraction (LF), occluded light fraction (LF), total heavy fraction (HF)) and bulk soil. Means for all CO₂ concentration and nitrogen deposition treatments are shown due to lack of significant treatment effects ($p = 0.05$).

Supplementary Table 2

Molecular proxies (carbon preference index, CPI; average chain length, ACL) of total (C₂₀₋₃₄), mid- (C₂₀₋₂₇) and long chain (C₂₇₋₃₄) *n*-alkanes in plant biomass (beech leaves, spruce needles, beech roots, spruce roots), soil density fractions (free light fraction (LF), occluded light fraction (LF), total heavy fraction (HF)) and bulk soil. Means for all CO₂ concentration and nitrogen deposition treatments are shown due to lack of significant treatment effects ($p = 0.05$).

Supplementary Table 3

Stable carbon isotope values ($\delta^{13}\text{C}$) of mid- (C₂₀₋₂₇) and long chain (C₂₇₋₃₄) *n*-alkanes in plant biomass (beech leaves, spruce needles, beech roots, spruce roots) under treatments with ambient and ¹³C-depleted CO₂ concentration in combination with low and high nitrogen (N) deposition. $\delta_{\text{ambient}} - \delta_{\text{depleted}}$

represents the difference in $\delta^{13}\text{C}$ between treatments with ambient and ^{13}C -depleted CO_2 for low and high N deposition.

Supplementary Table 4

Stable carbon isotope values ($\delta^{13}\text{C}$) of mid- (C_{20-27}) and long chain (C_{27-34}) *n*-alkanes in soil density fractions (free light fraction (LF), occluded light fraction (LF), total heavy fraction (HF)) and bulk soil under treatments with ambient and ^{13}C -depleted CO_2 concentration in combination with low and high nitrogen (N) deposition. $\delta_{\text{ambient}} - \delta_{\text{depleted}}$ represents the difference in $\delta^{13}\text{C}$ between treatments with ambient and ^{13}C -depleted CO_2 for low and high N deposition.

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Supplementary table 2

Table 2

Molecular proxies (carbon preference index, CPI; average chain length, ACL) of total (C_{20-34}), mid- (C_{20-27}) and long-chain (C_{27-34}) *n*-alkanes in plant biomass (beech leaves, spruce needles, beech roots, spruce roots), soil density fractions (free light fraction (LF), occluded light fraction (LF), total heavy fraction (HF)) and bulk soil. Means for all CO_2 concentration and nitrogen deposition treatments are shown due to lack of significant treatment effects ($p = 0.05$).

	plant biomass				soil density fractions			
	beech leaves	spruce needles	beech roots	spruce roots	free LF	occluded LF	total HF	bulk soil
total <i>n</i> -alkanes (C ₂₀₋₃₄)								
% C ₂₇ ^a	82 ± 1	15 ± 1	14 ± 1	11 ± 0	18 ± 1	17 ± 1	27 ± 2	27 ± 2
% odd ^b	95 ± 0	58 ± 2	50 ± 1	49 ± 1	58 ± 2	56 ± 1	73 ± 2	73 ± 2
% even ^c	5 ± 0	42 ± 2	50 ± 1	51 ± 1	42 ± 2	44 ± 1	27 ± 2	27 ± 2
CPI ^d	17.9 ± 0.6	1.5 ± 0.1	1.1 ± 0.0	1.0 ± 0.0	1.5 ± 0.2	1.3 ± 0.1	3.0 ± 0.2	3.1 ± 0.3
ACL ^e	27.1 ± 0.0	26.9 ± 0.1	25.7 ± 0.1	25.8 ± 0.1	26.2 ± 0.3	26.0 ± 0.1	27.9 ± 0.1	28.2 ± 0.2
mid-chain <i>n</i> -alkanes (C ₂₀₋₂₇)								
% C ₂₇ ^f	93 ± 0	25 ± 1	20 ± 1	16 ± 0	27 ± 2	24 ± 1	52 ± 1	56 ± 1
% odd ^g	96 ± 0	55 ± 2	51 ± 1	49 ± 1	56 ± 2	55 ± 1	75 ± 1	79 ± 1
% even ^h	4 ± 0	45 ± 2	49 ± 1	51 ± 1	44 ± 2	45 ± 1	25 ± 1	21 ± 1
CPI ⁱ	20.9 ± 0.7	1.1 ± 0.1	1.0 ± 0.0	0.9 ± 0.0	1.2 ± 0.1	1.2 ± 0.0	2.7 ± 0.2	3.2 ± 0.1
ACL ^j	26.8 ± 0.0	24.8 ± 0.1	24.2 ± 0.1	24.1 ± 0.1	24.5 ± 0.1	24.6 ± 0.1	25.5 ± 0.0	25.7 ± 0.0
long-chain <i>n</i> -alkanes (C ₂₇₋₃₄)								
% C ₂₇ ^k	88 ± 1	27 ± 1	34 ± 1	28 ± 1	38 ± 3	38 ± 1	36 ± 2	34 ± 3
% odd ^l	98 ± 0	72 ± 2	65 ± 1	62 ± 1	76 ± 2	73 ± 1	81 ± 3	79 ± 3
% even ^m	2 ± 0	28 ± 2	35 ± 1	38 ± 1	24 ± 2	27 ± 1	19 ± 3	21 ± 3
CPI ⁿ	35.6 ± 1.5	2.1 ± 0.1	1.5 ± 0.1	1.3 ± 0.1	2.7 ± 0.3	2.2 ± 0.1	4.3 ± 0.4	4.0 ± 0.5
ACL ^o	27.2 ± 0.0	29.1 ± 0.1	28.8 ± 0.0	29.0 ± 0.1	28.9 ± 0.2	28.7 ± 0.1	29.2 ± 0.1	29.3 ± 0.1
long-chain / mid-chain								
odd ^p	1.1 ± 0.0	1.2 ± 0.1	0.7 ± 0.0	0.7 ± 0.0	1.0 ± 0.1	0.8 ± 0.0	1.6 ± 0.1	1.7 ± 0.1
even ^q	0.5 ± 0.0	0.6 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	1.2 ± 0.2	1.9 ± 0.4
total ^r	1.1 ± 0.0	0.9 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.8 ± 0.1	0.6 ± 0.0	1.5 ± 0.1	1.8 ± 0.1

[illegible]

Supplementary table 3

Table 3

Stable carbon isotope values ($\delta^{13}\text{C}$) of mid- (C_{20-27}) and long-chain (C_{27-34}) *n*-alkanes in plant biomass (beech leaves, spruce needles, beech roots, spruce roots) under treatments with ambient and ^{13}C -depleted CO_2 concentration in combination with low and high nitrogen (N) deposition. $\delta_{\text{ambient}} - \delta_{\text{depleted}}$ represents the difference in $\delta^{13}\text{C}$ between treatments with ambient and ^{13}C -depleted CO_2 for low and high N deposition.

	CO₂ concentration	N deposition	mid-chain (C₂₀₋₂₇)	long-chain (C₂₇₋₃₄)
			$\delta^{13}\text{C}$ (‰)	$\delta^{13}\text{C}$ (‰)
beech leaves	ambient	low	-28.6 ± 0.1	-28.7 ± 0.1
	ambient	high	-28.4 ± 0.1	-28.4 ± 0.1
	^{13}C -depleted	low	-40.7 ± 0.3	-40.8 ± 0.3
	^{13}C -depleted	high	-37.8 ± 0.1	-37.9 ± 0.1
	$\delta_{\text{ambient}} - \delta_{\text{depleted}}$	low	12.1 ± 0.3	12.1 ± 0.3
	$\delta_{\text{ambient}} - \delta_{\text{depleted}}$	high	9.4 ± 0.2	9.5 ± 0.2
spruce needles	ambient	low	-27.3 ± 0.2	-28.1 ± 0.2
	ambient	high	-27.0 ± 0.1	-27.4 ± 0.1
	^{13}C -depleted	low	-29.0 ± 0.1	-31.3 ± 0.1
	^{13}C -depleted	high	-29.2 ± 0.2	-31.4 ± 0.1
	$\delta_{\text{ambient}} - \delta_{\text{depleted}}$	low	1.7 ± 0.2	3.2 ± 0.2
	$\delta_{\text{ambient}} - \delta_{\text{depleted}}$	high	2.2 ± 0.2	4.0 ± 0.1
beech roots	ambient	low	-27.5 ± 0.2	-27.0 ± 0.3
	ambient	high	-27.5 ± 0.1	-28.2 ± 0.2
	^{13}C -depleted	low	-27.7 ± 0.4	-28.4 ± 0.4
	^{13}C -depleted	high	-27.7 ± 0.4	-28.4 ± 0.5
	$\delta_{\text{ambient}} - \delta_{\text{depleted}}$	low	0.2 ± 0.4	1.3 ± 0.5
	$\delta_{\text{ambient}} - \delta_{\text{depleted}}$	high	0.2 ± 0.4	0.2 ± 0.5
spruce roots	ambient	low	-27.4 ± 0.2	-27.5 ± 0.1
	ambient	high	-27.1 ± 0.2	-27.7 ± 0.2
	^{13}C -depleted	low	-27.4 ± 0.2	-27.3 ± 0.2
	^{13}C -depleted	high	-28.8 ± 0.8	-29.5 ± 1.4
	$\delta_{\text{ambient}} - \delta_{\text{depleted}}$	low	0.1 ± 0.3	-0.2 ± 0.2
	$\delta_{\text{ambient}} - \delta_{\text{depleted}}$	high	1.7 ± 0.8	1.8 ± 1.5

Supplementary table 4

Table 4

Stable carbon isotope values ($\delta^{13}\text{C}$) of mid- (C_{20-27}) and long-chain (C_{27-34}) *n*-alkanes in soil density fractions (free light fraction (LF), occluded light fraction (LF), total heavy fraction (HF)) and bulk soil under treatments with ambient and ^{13}C -depleted CO_2 concentration in combination with low and high nitrogen (N) deposition. $\delta_{\text{ambient}} - \delta_{\text{depleted}}$ represents the difference in $\delta^{13}\text{C}$ between treatments with ambient and ^{13}C -depleted CO_2 for low and high N deposition

	CO₂ concentration	N deposition	mid-chain (C₂₀₋₂₇)	long-chain (C₂₇₋₃₄)
			$\delta^{13}\text{C}$ (‰)	$\delta^{13}\text{C}$ (‰)
free LF	ambient	low	-27.7 ± 0.1	-29.9 ± 0.2
	ambient	high	-27.5 ± 0.1	-28.9 ± 0.1
	^{13}C -depleted	low	-28.7 ± 0.2	-33.1 ± 0.6
	^{13}C -depleted	high	-28.2 ± 0.2	-31.2 ± 0.3
	$\delta_{\text{ambient}} - \delta_{\text{depleted}}$	low	1.0 ± 0.2	3.1 ± 0.6
	$\delta_{\text{ambient}} - \delta_{\text{depleted}}$	high	0.7 ± 0.2	2.3 ± 0.3
occluded LF	ambient	low	-27.5 ± 0.1	-28.6 ± 0.2
	ambient	high	-27.3 ± 0.1	-27.9 ± 0.1
	^{13}C -depleted	low	-27.5 ± 0.1	-29.0 ± 0.2
	^{13}C -depleted	high	-27.3 ± 0.1	-28.4 ± 0.1
	$\delta_{\text{ambient}} - \delta_{\text{depleted}}$	low	0.1 ± 0.2	0.4 ± 0.2
	$\delta_{\text{ambient}} - \delta_{\text{depleted}}$	high	0.0 ± 0.1	0.5 ± 0.1
total HF	ambient	low	-28.5 ± 0.1	-30.3 ± 0.1
	ambient	high	-28.7 ± 0.1	-30.6 ± 0.0
	^{13}C -depleted	low	-28.9 ± 0.1	-31.9 ± 0.2
	^{13}C -depleted	high	-29.1 ± 0.1	-30.4 ± 0.3
	$\delta_{\text{ambient}} - \delta_{\text{depleted}}$	low	0.4 ± 0.1	1.6 ± 0.2
	$\delta_{\text{ambient}} - \delta_{\text{depleted}}$	high	0.4 ± 0.1	-0.2 ± 0.3
bulk soil	ambient	low	-28.8 ± 0.0	-30.5 ± 0.2
	ambient	high	-28.6 ± 0.1	-30.1 ± 0.1
	^{13}C -depleted	low	-29.4 ± 0.1	-31.4 ± 0.1
	^{13}C -depleted	high	-29.3 ± 0.1	-30.3 ± 0.3
	$\delta_{\text{ambient}} - \delta_{\text{depleted}}$	low	0.6 ± 0.1	1.0 ± 0.2
	$\delta_{\text{ambient}} - \delta_{\text{depleted}}$	high	0.7 ± 0.2	0.2 ± 0.3