Oecologia (2015) 178:981–998
DOI 10.1007/s00442-015-3278-6



PHYSIOLOGICAL ECOLOGY - ORIGINAL RESEARCH

# Concentrations and $\delta^2H$ values of cuticular *n*-alkanes vary significantly among plant organs, species and habitats in grasses from an alpine and a temperate European grassland

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Received: 22 October 2014 / Accepted: 17 February 2015 / Published online: 13 March 2015


**Abstract** *n*-Alkanes are long-chained hydrocarbons contained in the cuticle of terrestrial plants. Their hydrogen isotope ratios ($\delta^2H$) have been used as a proxy for environmental and plant ecophysiological processes. Calibration studies designed to resolve the mechanisms that determine the $\delta^2H$ values of *n*-alkanes have exclusively focused on *n*-alkanes derived from leaves. It is, however, unclear in which quantities *n*-alkanes are also produced by other plant organs such as roots or inflorescences, or whether different plant organs produce distinct *n*-alkane $\delta^2H$ values. To resolve these open questions, we sampled leaves, sheaths, stems, inflorescences and roots from a total of 15 species of European C3 grasses in an alpine and a temperate grassland in Switzerland. Our data show slightly increased *n*-alkane concentrations and *n*-alkane $\delta^2H$ values in the alpine compared to the temperate grassland. More importantly, inflorescences had typically much higher *n*-alkane concentrations than other organs while roots had very low *n*-alkane concentrations. Most interestingly, the $\delta^2H$ values of the carbon autonomous plant organs leaves, sheaths and stems were in general depleted compared to the overall mean $\delta^2H$ value of a species, while non-carbon autonomous organs such as roots and inflorescences show $\delta^2H$ values that are higher compared to the overall mean $\delta^2H$ value of a species. We attribute organ-specific $\delta^2H$ values to differences in the H-NADPH biosynthetic origin in different plant organs as a function of their carbon relationships. Finally, we employed simple mass balance calculations to show that leaves are in fact the main source of *n*-alkanes in the sediment. As such, studies assessing the environmental and physiological drivers of *n*-alkanes that focus on leaves produce relationships that can be employed to interpret the $\delta^2H$ values of *n*-alkanes derived from sediments. This is despite the significant differences that we found among the $\delta^2H$ values in the different plant organs. Our study brings new insights into the natural variability of *n*-alkane $\delta^2H$ values and has implications for the interpretation of *n*-alkane $\delta^2H$ values in ecological and paleohydrological research.

**Keywords** Ecophysiology · Paleoecology · *n*-Alkanes · Stable isotopes · Grass organs

Communicated by Miquel Gonzalez-Meler.

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## Introduction

H, C and O isotope ratios of plant-originated material correlate with environmental variables such as temperature and precipitation (Craig and Gordon 1965; Dawson and Ehleringer 1993). The stable isotope composition of plant materials has thus been used with success to investigate environmental and physiological processes (Dawson et al. 2002). While oxygen and in particular carbon isotope analysis of plant materials are now widely applied in plant ecological research, the hydrogen isotope composition of plants is rarely considered. This is because of methodological limitations and the difficulty of analyzing hydrogen isotopes of bulk plant materials with sufficient accuracy. In particular, oxygen-bound hydrogen atoms, e.g., in cellulose, can rapidly exchange with hydrogen from ambient moisture, making it difficult to analyze and interpret hydrogen isotopes in plants on a routine basis.

With the introduction of compound-specific stable isotope analyses, it is now possible to determine the hydrogen

isotope composition ($\delta^2H$) of defined compounds, such as leaf wax lipids and, in particular, leaf wax *n*-alkanes (Burgoyne and Hayes 1998; Hilkert et al. 1999). These compounds are hydrocarbons that are synthesized in the cuticle of terrestrial plants (Buschhaus et al. 2007; Eglinton and Eglinton 2008). In these compounds, all hydrogen atoms are covalently bound to carbon atoms and thus do not exchange with their environment. As such, exchangeable hydrogen atoms do not hinder the interpretation of $\delta^2H$ signals in these compounds and open the door to new applications of stable hydrogen isotopes in ecological and environmental research. In addition, *n*-alkanes have low degradation rates and their $\delta^2H$ values remain unchanged during sedimentary diagenesis. *n*-Alkanes therefore accumulate in soil and sediments (Eglinton and Eglinton 2008), and have thus been celebrated as stable and powerful biomarkers for contemporary ecological and paleoclimatological research (Sachse et al. 2012).

Sachse et al. (2004) found a linear relationship between $\delta^2H$ values of meteoric water and plant-derived *n*-alkanes $\delta^2H$ values of different plant types across a European north–south transect. Based on this relationship, it has been suggested that *n*-alkane $\delta^2H$ values could act as proxy for precipitation $\delta^2H$ values. However, large scatter is typically observed when plant-derived *n*-alkane $\delta^2H$ values are correlated with precipitation $\delta^2H$ values across environmental gradients (Sachse et al. 2004, 2012; Polissar and Freeman 2010; Garcin et al. 2012). This suggests that, in addition to precipitation $\delta^2H$ values, other factors also influence the $\delta^2H$ values of *n*-alkanes. Over the past years, there have been increasing research efforts to resolve the drivers of this scatter. In summary, three major drivers of *n*-alkane $\delta^2H$ values have been identified: source water $\delta^2H$ values (Sauer et al. 2001; Liu et al. 2006), leaf water evaporative $^2H$-enrichment (Smith and Freeman 2006; Kahmen et al. 2013a, b), and biosynthetic fractionation (Chikaraishi et al. 2004). Based on this conceptual framework, a better interpretation of plant-derived *n*-alkane $\delta^2H$ values, e.g., from sediment records, is now possible.

Most research efforts designed to resolve and quantify the mechanisms that determine the amount and $\delta^2H$ values of *n*-alkanes have focused on *n*-alkanes derived from leaves. In contrast, few studies have analyzed whether other plant organs produce *n*-alkanes in amounts that are comparable to those in leaves. Since it has recently been suggested that the majority of sediment or soil *n*-alkane could derive from roots rather than from leaves (Kuhn et al. 2010; Gocke et al. 2011; Huang et al. 2011), it remains unclear whether sediment derived *n*-alkanes are truly "leaf waxes" or rather "whole plant waxes". What is more, there is insufficient information on whether the drivers of *n*-alkane $\delta^2H$ values that have now been described for leaves also apply to *n*-alkane $\delta^2H$ values derived from other plant organs such as sheaths, stems, inflorescences or roots. We know from previous studies that there can be significant differences in carbon isotope ratios among different plant organs (e.g., Cernusak et al. 2009). Early work by Ziegler et al. (1976) on plant bulk hydrogen isotope composition indicated that similar within plant variability could also be true for $\delta^2H$ values. As such, studies assessing the mechanistic drivers of *n*-alkane $\delta^2H$ values need to be expanded from being solely leaf focused to include other plant organs in order to determine whether systematic within plant variability of *n*-alkane concentrations and $\delta^2H$ values exists and to improve our interpretation of $\delta^2H$ values of plant-derived compounds in the environment.

We have investigated the concentration and $\delta^2H$ values of *n*-alkanes in different plant organs such as leaves, stems, inflorescences and roots of 15 different temperate and alpine C3 grasses that grew in two contrasting environments in Switzerland. The goal of our study was (1) to assess whether *n*-alkanes are synthesized primarily in the cuticle of leaves or whether other plant organs such as roots or inflorescences also produce large amounts of *n*-alkanes as has been recently suggested, and (2) to assess whether systematic differences exist in the hydrogen isotope composition of *n*-alkanes derived from different plant organs. With these two objectives, our study aims to contribute to a more robust interpretation of *n*-alkane $\delta^2H$ values in plants and sedimentary records.

## Materials and methods

### Sampling

To determine the concentrations and $\delta^2H$ values in different plant organs, we sampled a total of 15 European C3 grass species that grew either in an alpine or a temperate grassland in Switzerland. We selected the two sites based on their differences in environmental conditions and their partly overlapping species composition: The study sites were at Alp Weissenstein, Graubünden, for the alpine grassland and at Ennetbaden, Aargau, for the temperate grassland. The research station Alp Weissenstein is located at 46°34′N, 9°47′E and an elevation of 1978 m above sea level (m.a.s.l.). The growing season at this location lasts from mid-June to September. The annual precipitation is 918 mm and the mean annual temperature 2.3 °C. The research site at Ennetbaden is located at 47°29′N, 8°19′E and at an elevation of 360 m.a.s.l. The growing season lasts from March to October. The annual precipitation is 1057 mm, and mean annual temperature 9 °C.

We sampled the most abundant species at each site. From the alpine grassland, these were: *Dactylis glomerata, Poa alpina, Phleum raeticum, Festuca rubra, Nardus*

*stricta, Briza media, Sesleria caerulea* and *Deschampsia cespitosa*. From the temperate grassland, we sampled *Brachypodium pinnatum, Arrhenatherum elatius, Dactylis glomerata, Festuca rubra, Phleum pratense, Lolium perenne and Lolium multiflorum*. For each species, about 20–30 individuals were collected and bulked in order to obtain sufficient plant material. All individuals of a species that we collected grew within a proximity of 20 m. The total sampling area was of about 50 × 50 m for Ennetbaden and of about 200 × 200 m for Alp Weissenstein. To assure plants were fully matured, they were collected during the peak growing season at both sites (at Ennetbaden, 27 June 2012 and at Alp Weissenstein, 22 July 2013). After their collection, plant material was dried for 24 h at 60 °C in a drying oven. Fertile grass tillers were then divided into their five main organs: leaves, stems, sheaths, roots and inflorescences, while infertile tillers without inflorescences were sectioned into vegetative leaves, vegetative stems and vegetative sheaths. We estimated that about 10–50 % of the tillers were fertile, with high variability across species.

## Lipid purification

After being separated into different organs, approximately 1 g of dried plant material was clipped into small parts for lipid extraction and purification. Clipped plant parts were transferred into a 40-ml beaker. Total lipids were extracted (TLE) by addition of 30 ml of a mixture of dichloromethane (DCM) and methanol (9:1) and placing the beaker in an ultrasonic bath for 15 min (Peters et al. 2005; Christie and Han 2010). This extraction was done to separate the highly apolar lipids, which include molecules such as alkyls (including *n*-alkanes), fatty acids, alcohols and esters from other polar components of the plant material, e.g., chlorophyll or sugars. The long-chained *n*-alkanes were further purified from other apolar molecules using liquid chromatography (LC). To do so we used 6 ml glass columns that were sterilized in a combustion oven at 500 °C for 5 h. Then, 3/4 vol of the columns were packed with silica gel 60 (0.040–0.063 mm Alfa Aesar; Johnson Matthey) 99.5 % pure. The packed columns were rinsed with two volumes of acetone, two volumes DCM and two volumes of hexane, then the silica gel columns were temperature-activated and dried in a desiccation oven at 60 °C overnight. To obtain the fraction containing *n*-alkanes (fraction 1), the TLE was passed through the silica gel columns with 12 ml of *n*-hexane (GC grade). Thereafter, a mixture of hexane and DCM (1:1) was passed through the remaining column to obtain the fatty acids (fraction 2). The remaining apolar material (fraction 3) was obtained by passing a mixture of DCM and methanol (9:1) through the column. For the results reported here, only fraction 1 was considered.

## *n*-Alkane concentration and isotopic analysis

*n*-Alkanes in fraction 1 were identified and quantified using a gas chromatograph (GC; 7890A; Agilent Technologies) coupled with a flame ionization detector (FID) at the Geologisches Institut, ETH Zurich. Measurements were done on a 30-m column (DB-5; Agilent) with a diameter of 0.250 mm and film thickness of 0.25 μm. The compound quantification was done by peak area comparison with an internal standard (α-androstane). *n*-Alkane concentrations for entire plants or specific organs are reported throughout this manuscript as μg/g biomass.

We calculated the average chain length (ACL) for each sample to compare the preference of plant organs to synthesize a length of alkane (short or long) as:

$$\mathrm{ACL} = \Sigma nxC / \Sigma C \quad (1)$$

where $n$ is the carbon chain-length (odd only and considering carbon lengths from $nC_{25}$ to $nC_{35}$) and $C$ is the concentration of alkane with $n$ carbons.

The hydrogen isotope composition of *n*-alkanes was measured with an isotope ratio mass spectrometer (IRMS; Delta V Plus; ThermoFisher) coupled to a GC (Trace GC Ultra; ThermoFisher). Compounds were separated in the GC on a 30-m column (DB-5; Agilent) with a diameter of 0.250 mm and film thickness of 0.25 μm. The injector was in splitless mode at a temperature of 270 °C. Next, 1 μl of each sample with a concentration of 300 ng/μl of the most abundant *n*-alkane was injected in triplicates. During sample separation, the GC oven temperature was first held at 90 °C for 2 min, then raised to 150 °C at 10 °C per min, it finally reached 320 °C at a rate of 4 °C per min. This temperature was held for 10 min. After separation on the GC column, individual compounds were converted to $H_2$ gas in an aluminum oxide reactor at 1420 °C. In each sequence, we ran ten samples in triplicate injections. An *n*-alkane standard mixture (A4, provided by A. Schimmelmann, Indiana University) was run at three different concentrations (100, 200, 400 ng/μl) for three times during each sequence. In total, this resulted in 42 injections per sequence. Injecting standards in different concentrations revealed that peak sizes below an area of 20 Vs produced unstable $\delta^2H$ values. As such, sample compounds with peak sizes smaller than 20 Vs were omitted from the analyses. The linear relationship of known and measured $\delta^2H$ values from the A4 mixture was used to derive sample $\delta^2H$ values relative to the VSMOW scale. The precision of the instrument was assessed on the basis of an internal laboratory standard ($nC_{29}$ alkane from oak leaves) which was measured in all the sequences; its mean standard deviation for all sequences analyses in this study was 9.4 permille. The $H3^+$ factor was calculated at the beginning of each sequence and had a mean value of 2.5 during the analyses.

### Data analysis and statistics

For each plant organ of a species, only a single sample consisting of tissue from >20 bulked tillers was analyzed. The reason for bulking the samples of a species was the labor-intensive sample preparation and isotope analysis and the fact that the organ of a single individual would not have yielded sufficient amounts of *n*-alkanes for isotopic analyses. The main experimental unit of our work was thus the "plant organ", with the individual species that we sampled serving as replicates in the statistical analyses (for details, see below). To compare $\delta^2$H values of plant organs across species, we determined the concentration weighed mean $\delta^2$H values of $n$C$_{29}$ and $n$C$_{31}$ for each organ of a species. $n$C$_{29}$ and $n$C$_{31}$ turned out to be the most abundant compounds across the investigated species and allowed reliable hydrogen isotope ratio measurements. To test for significant differences in *n*-alkane concentrations and $\delta^2$H values across plant organs and between research sites, we performed an analysis of variance (ANOVA) with organs and research sites as factors.

To assess whether plant organs systematically deviate in their $\delta^2$H values from the plants' mean $\delta^2$H values in a site, we estimated for each species at each site a "plant mean $\delta^2$H value" by averaging the $\delta^2$H values of all organs for a species at a site. We then calculated the deviation of *n*-alkane $\delta^2$H values from this mean for each organ of a species. To detect general across-species trends in organ $\delta^2$H values, we estimated the mean of these deviations for each organ across species at a site. This standardization procedure was necessary because across-species variability in $\delta^2$H values can be large and exceed within-plant variability. To test for significant differences in organs' *n*-alkane $\delta^2$H deviation from the species mean, we performed an ANOVA for each of the two sites with organ as factor followed by a Fisher's Least Significant Differences (LSD) test. Testing interactions between species and site or organs were not possible due to lack of replication at the species level. All statistical calculations were done using the statistical package R v.3.1.1 (http://www.r-project.org).

## Results

### *n*-Alkane composition and concentration

We found that grasses at Alp Weissenstein and Ennetbaden had a strong preference for odd over even long-chained *n*-alkanes, with chains containing 25–33 carbon atoms being the most common (Table 1; Figs. 1, 2). Among these, $n$C$_{29}$ and $n$C$_{31}$ were generally the most abundant *n*-alkanes at both sites and the sum of $n$C$_{29}$ or $n$C$_{31}$ among species was always higher than any other $n$C length, except for *P. pratense* at Ennetbaden, where the contribution from $n$C$_{27}$ was also important (Table 1; Fig. 2). The ACL for all green organs (leaves, sheaths, stems and vegetative organs) surpasses 30 across all species whereas inflorescences and roots had a species averaged ACL of 28.8 and 29.6, respectively (Table 1).

We found that total *n*-alkane concentrations varied significantly among species and organs and were marginally significant between sites (Tables 1, 2; Figs. 1, 2). In general, plants had higher *n*-alkane concentrations at the alpine site Alp Weissenstein where they ranged from 128 to 891 μg/g (Table 1) as compared to the temperate site at Ennetbaden, where total *n*-alkane concentration ranged from 64 to 199 μg/g. Among organs, there was a significant trend across species and sites that inflorescences had the largest *n*-alkane concentration whereas roots had the lowest amount of *n*-alkanes. At Alp Weissenstein, the average inflorescence *n*-alkane concentration was 848 μg/g, while roots had an average concentration of 65 μg/g. Other green organs varied greatly: leaves had concentrations that varied between 153 and 1083 μg/g (Table 1; Fig. 1). At Ennetbaden, the average *n*-alkane concentration for inflorescence was 460 μg/g, while roots had an across-species average concentration of only 17 μg/g and leaves varied between 33 and 219 μg/g (Table 1; Fig. 2).

### *n*-Alkane $\delta^2$H values

Concentration-weighted ($n$C$_{29}$–$n$C$_{31}$) plant-averaged (across organs of a species) *n*-alkane $\delta^2$H values ranged from −266 to −231 ‰ at Alp Weissenstein and from −253 to −199 ‰ at Ennetbaden for the investigated species (Table 3; Figs. 3, 4). In general, *n*-alkane $\delta^2$H values were more depleted in plants growing at Alp Weissenstein than at Ennetbaden (Table 4). We found significant across-organ variability in *n*-alkane $\delta^2$H values which was up to 102 ‰ (e.g., *D. cespitosa*) within a species (Fig. 3; Tables 3, 4). In general, inflorescences and roots carried more positive $\delta^2$H values than other organs: At Alp Weissenstein, mean $\delta^2$H *n*-alkane values of inflorescences and roots were −213 and −228 ‰ compared to −254 ‰ in leaves. At Ennetbaden, inflorescences and roots had $\delta^2$H values of −204 and −218 ‰ compared to −229 ‰ in leaves. As such, we observed a general trend for green and carbon autonomous plant organs such as leaves to have more negative $\delta^2$H values than organs of the same species that are not carbon autonomous such as inflorescences and roots. This pattern was consistent for both sites and became even more evident, when we calculated the deviation of an individual organ $\delta^2$H value from a species mean $\delta^2$H value (Table 4; Fig. 5). Across all species in a site, the $\delta^2$H values of green organs such as leaves, sheaths and stems, were in general depleted in $^2$H compared to the plants' mean $\delta^2$H values (Fig. 5). This was in contrast to inflorescences and roots, which showed always significantly $^2$H enriched $\delta^2$H values. When averaged for each site, we found that $\delta^2$H values of

**Table 1** *n*-Alkane concentrations (μg/g) and ACL for different organs from grasses at Alp Weissenstein and Ennetbaden

| Alp Weisenstein | $C_{25}$ | $C_{27}$ | $C_{29}$ | $C_{31}$ | $C_{33}$ | $C_{25}$–$C_{33}$ | ACL | Ennetbaden | $C_{25}$ | $C_{27}$ | $C_{29}$ | $C_{31}$ | $C_{33}$ | $C_{25}$–$C_{33}$ | ACL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *D. glomerata* | | | | | | | | *B. pinnatum* | | | | | | | |
| Leaves | 16 | 13 | 38 | 56 | 30 | 153 | 29.9 | Leaves | 0 | 9 | 10 | 25 | 4 | 48 | 30.0 |
| Sheath | 13 | 12 | 61 | 48 | 10 | 142 | 29.4 | Sheath | 0 | 3 | 17 | 41 | 4 | 66 | 30.4 |
| Stem | 3 | 3 | 9 | 9 | 2 | 25 | 29.3 | Stem | 0 | 1 | 6 | 12 | 0 | 19 | 30.2 |
| Veg. leaves | 7 | 7 | 16 | 30 | 20 | 80 | 30.2 | Veg. leaves | 2 | 11 | 15 | 39 | 6 | 73 | 30.0 |
| Veg. sheath | 13 | 4 | 9 | 18 | 13 | 57 | 29.5 | Veg. sheath | 0 | 8 | 25 | 52 | 5 | 90 | 30.2 |
| Veg. stem | 2 | 1 | 3 | 3 | 1 | 9 | 28.8 | Veg. stem | 0 | 0 | 7 | 13 | 1 | 20 | 30.5 |
| Inflorescence | 115 | 130 | 143 | 98 | 23 | 509 | 28.1 | Inflorescence | 31 | 107 | 60 | 51 | 7 | 256 | 28.2 |
| Roots | 3 | 11 | 9 | 12 | 13 | 48 | 29.9 | Roots | 0 | 1 | 1 | 1 | 0 | 3 | 29.3 |
| Species mean | | | | | | 128 | 29.4 | Species mean | | | | | | 72 | 29.9 |
| *P. alpina* | | | | | | | | *A. elatius* | | | | | | | |
| Leaves | 20 | 8 | 13 | 44 | 117 | 202 | 31.3 | Leaves | 23 | 10 | 26 | 69 | 82 | 210 | 30.7 |
| Sheath | 6 | 7 | 24 | 72 | 55 | 163 | 31.0 | Sheath | 12 | 12 | 14 | 37 | 51 | 126 | 30.6 |
| Stem | 1 | 3 | 17 | 41 | 10 | 72 | 30.5 | Stem | 3 | 4 | 29 | 44 | 4 | 83 | 30.0 |
| Veg. leaves | 23 | 9 | 14 | 36 | 91 | 173 | 30.9 | Veg. leaves | 15 | 7 | 17 | 49 | 62 | 150 | 30.8 |
| Veg. sheath | 5 | 10 | 27 | 46 | 25 | 113 | 30.3 | Veg. sheath | 15 | 15 | 39 | 71 | 37 | 176 | 30.1 |
| Veg. stem | 8 | 13 | 46 | 110 | 31 | 208 | 30.4 | Veg. stem | 3 | 6 | 29 | 54 | 6 | 98 | 30.1 |
| Inflorescence | 93 | 101 | 133 | 151 | 60 | 538 | 28.9 | Inflorescence | 28 | 56 | 114 | 141 | 42 | 380 | 29.6 |
| Roots | 5 | 6 | 12 | 19 | 9 | 51 | 29.8 | Roots | 3 | 3 | 3 | 5 | 4 | 17 | 29.4 |
| Species mean | | | | | | 190 | 30.4 | Species mean | | | | | | 155 | 30.2 |
| *P. raeticum* | | | | | | | | *D. glomerata* | | | | | | | |
| Leaves | 18 | 9 | 24 | 88 | 48 | 188 | 30.5 | Leaves | 19 | 11 | 18 | 34 | 26 | 108 | 29.7 |
| Sheath | 9 | 9 | 54 | 121 | 23 | 217 | 30.3 | Sheath | 11 | 7 | 16 | 23 | 6 | 64 | 29.2 |
| Stem | 2 | 3 | 46 | 92 | 5 | 148 | 30.3 | Stem | 4 | 2 | 7 | 10 | 2 | 25 | 29.2 |
| Veg. leaves | 11 | 8 | 33 | 118 | 47 | 217 | 30.7 | Veg. leaves | 13 | 7 | 13 | 28 | 15 | 75 | 29.7 |
| Veg. sheath | 3 | 4 | 21 | 32 | 8 | 68 | 30.1 | Veg. sheath | 26 | 13 | 12 | 9 | 5 | 65 | 27.5 |
| Veg. stem | 2 | 4 | 32 | 61 | 8 | 107 | 30.3 | Veg. stem | 34 | 9 | 5 | 3 | 3 | 54 | 26.5 |
| Inflorescence | 165 | 208 | 262 | 185 | 29 | 849 | 28.3 | Inflorescence | 217 | 244 | 271 | 339 | 92 | 1163 | 28.7 |
| Roots | 1 | 4 | 6 | 9 | 6 | 26 | 30.1 | Roots | 3 | 14 | 10 | 5 | 3 | 34 | 28.5 |
| Species mean | | | | | | 228 | 30.1 | Species mean | | | | | | 199 | 28.6 |
| *F. rubra* | | | | | | | | *F. rubra* | | | | | | | |
| Leaves | 15 | 13 | 157 | 324 | 118 | 626 | 30.6 | Leaves | 6 | 9 | 117 | 71 | 16 | 219 | 29.7 |
| Sheath | 3 | 11 | 325 | 406 | 103 | 849 | 30.4 | Sheath | 3 | 8 | 156 | 62 | 4 | 233 | 29.5 |
| Stem | 1 | 5 | 90 | 84 | 22 | 202 | 30.2 | Stem | 1 | 4 | 51 | 15 | 1 | 72 | 29.3 |
| Veg. leaves | 14 | 15 | 120 | 328 | 128 | 603 | 30.8 | Veg. leaves | 3 | 5 | 51 | 49 | 7 | 116 | 29.9 |

**Table 1** continued

| Alp Weisenstein | $C_{25}$ | $C_{27}$ | $C_{29}$ | $C_{31}$ | $C_{33}$ | $C_{25}$–$C_{33}$ | ACL | Ennetbaden | $C_{25}$ | $C_{27}$ | $C_{29}$ | $C_{31}$ | $C_{33}$ | $C_{25}$–$C_{33}$ | ACL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Veg. sheath | 6 | 8 | 42 | 117 | 61 | 233 | 30.9 | Veg. sheath | 2 | 5 | 39 | 42 | 8 | 95 | 30.0 |
| Veg. stem | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | Veg. stem | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| Inflorescence | 127 | 180 | 205 | 199 | 31 | 742 | 28.5 | Inflorescence | 54 | 72 | 126 | 102 | 12 | 364 | 28.7 |
| Roots | 2 | 12 | 13 | 15 | 8 | 50 | 29.7 | Roots | 0 | 3 | 3 | 1 | 0 | 7 | 28.6 |
| Species mean | | | | | | 472 | 30.2 | Species mean | | | | | | 158 | 29.4 |
| *N. stricta* | | | | | | | | *P. pratense* | | | | | | | |
| Leaves | 5 | 11 | 133 | 204 | 62 | 415 | 30.5 | Leaves | 5 | 21 | 5 | 2 | 1 | 33 | 27.3 |
| Sheath | 8 | 27 | 352 | 787 | 292 | 1465 | 30.8 | Sheath | 3 | 18 | 4 | 1 | 0 | 26 | 27.3 |
| Stem | 13 | 28 | 333 | 815 | 292 | 1481 | 30.8 | Stem | 3 | 4 | 3 | 1 | 0 | 11 | 27.6 |
| Veg. leaves | 5 | 13 | 200 | 260 | 62 | 541 | 30.3 | Veg. leaves | 7 | 32 | 8 | 2 | 1 | 50 | 27.3 |
| Veg. sheath | 4 | 12 | 151 | 400 | 181 | 749 | 31.0 | Veg. sheath | 4 | 17 | 6 | 1 | 0 | 28 | 27.4 |
| Veg. stem | 4 | 9 | 112 | 293 | 110 | 527 | 30.9 | Veg. stem | 2 | 5 | 2 | 2 | 0 | 10 | 27.8 |
| Inflorescence | 34 | 108 | 406 | 562 | 116 | 1226 | 30.0 | Inflorescence | 55 | 94 | 82 | 74 | 6 | 312 | 28.2 |
| Roots | 3 | 31 | 31 | 30 | 11 | 107 | 29.3 | Roots | 0 | 1 | 21 | 23 | 0 | 44 | 30.0 |
| Species mean | | | | | | 814 | 30.5 | Species mean | | | | | | 64 | 27.9 |
| *B. media* | | | | | | | | *L. perenne* | | | | | | | |
| Leaves | 30 | 10 | 20 | 140 | 77 | 277 | 30.6 | Leaves | 3 | 6 | 29 | 53 | 25 | 116 | 30.5 |
| Sheath | 21 | 25 | 88 | 60 | 16 | 210 | 29.2 | Sheath | 0 | 5 | 69 | 129 | 14 | 216 | 30.4 |
| Stem | 12 | 11 | 26 | 13 | 3 | 65 | 28.5 | Stem | 0 | 1 | 13 | 22 | 2 | 38 | 30.3 |
| Veg. leaves | 47 | 18 | 52 | 107 | 41 | 265 | 29.6 | Veg. leaves | 0 | 5 | 31 | 56 | 31 | 123 | 30.8 |
| Veg. sheath | 113 | 72 | 251 | 389 | 80 | 905 | 29.6 | Veg. sheath | 0 | 5 | 50 | 95 | 20 | 170 | 30.5 |
| Veg. stem | 118 | 96 | 257 | 240 | 38 | 749 | 29.0 | Veg. stem | 0 | 6 | 27 | 44 | 12 | 89 | 30.4 |
| Inflorescence | 120 | 102 | 116 | 137 | 6 | 482 | 28.2 | Inflorescence | 57 | 80 | 67 | 113 | 10 | 327 | 28.6 |
| Roots | 7 | 13 | 23 | 43 | 15 | 102 | 29.9 | Roots | 0 | 1 | 2 | 2 | 1 | 6 | 30.3 |
| Species mean | | | | | | 382 | 29.3 | Species mean | | | | | | 136 | 30.3 |
| *S. caerulea* | | | | | | | | *L. multiforme* | | | | | | | |
| Leaves | 12 | 42 | 835 | 182 | 12 | 1083 | 29.3 | Leaves | 3 | 10 | 63 | 86 | 16 | 178 | 30.1 |
| Sheath | 10 | 40 | 3 | 488 | 96 | 637 | 31.0 | Sheath | 0 | 6 | 76 | 98 | 10 | 190 | 30.2 |
| Stem | 2 | 20 | 703 | 107 | 3 | 835 | 29.2 | Stem | 0 | 3 | 24 | 43 | 2 | 71 | 30.3 |
| Veg. leaves | 6 | 25 | 502 | 309 | 28 | 871 | 29.8 | Veg. leaves | 0 | 5 | 31 | 56 | 31 | 123 | 30.8 |
| Veg. sheath | 12 | 18 | 150 | 82 | 10 | 273 | 29.4 | Veg. sheath | 0 | 5 | 50 | 95 | 20 | 170 | 30.5 |
| Veg. stem | 5 | 18 | 328 | 311 | 38 | 700 | 30.0 | Veg. stem | 0 | 6 | 27 | 44 | 12 | 89 | 30.4 |
| Inflorescence | 21 | 68 | 292 | 147 | 157 | 685 | 30.0 | Inflorescence | 91 | 132 | 87 | 106 | 4 | 419 | 28.0 |
| Roots | 0 | 3 | 18 | 12 | 2 | 34 | 29.7 | Roots | 0 | 1 | 2 | 2 | 1 | 6 | 30.3 |
| Species mean | | | | | | 640 | 29.8 | Species mean | | | | | | 156 | 30.1 |

**Table 1** continued

| Alp Weisenstein | $C_{25}$ | $C_{27}$ | $C_{29}$ | $C_{31}$ | $C_{33}$ | $C_{25}$–$C_{33}$ | ACL | Ennetbaden | $C_{25}$ | $C_{27}$ | $C_{29}$ | $C_{31}$ | $C_{33}$ | $C_{25}$–$C_{33}$ | ACL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *D. cespitosa* | | | | | | | | | | | | | | | |
| Leaves | 15 | 15 | 134 | 325 | 97 | 587 | 30.6 | | | | | | | | |
| Sheath | 10 | 23 | 321 | 760 | 97 | 1211 | 30.5 | | | | | | | | |
| Stem | 14 | 28 | 395 | 1125 | 151 | 1714 | 30.6 | | | | | | | | |
| Veg. leaves | 53 | 38 | 266 | 602 | 246 | 1204 | 30.6 | | | | | | | | |
| Veg. sheath | 9 | 13 | 84 | 202 | 57 | 364 | 30.6 | | | | | | | | |
| Veg. stem | 6 | 9 | 47 | 104 | 21 | 187 | 30.3 | | | | | | | | |
| Inflorescence | 236 | 297 | 594 | 570 | 59 | 1756 | 28.9 | | | | | | | | |
| Roots | 6 | 8 | 25 | 48 | 16 | 104 | 30.2 | | | | | | | | |
| Species mean | | | | | | 891 | 30.3 | | | | | | | | |
| Mean values at Alp Weisenstein | | | | | | | | Mean values at Ennetbaden | | | | | | | |
| Leaves | 16 | 15 | 169 | 170 | 70 | 441 | 30.2 | Leaves | 8 | 11 | 38 | 49 | 24 | 131 | 30.1 |
| Sheath | 10 | 19 | 153 | 343 | 87 | 612 | 30.6 | Sheath | 4 | 8 | 50 | 56 | 13 | 132 | 30.0 |
| Stem | 6 | 13 | 202 | 286 | 61 | 568 | 30.3 | Stem | 2 | 2 | 19 | 21 | 2 | 46 | 29.8 |
| Veg. leaves | 21 | 17 | 150 | 224 | 83 | 494 | 30.3 | Veg. leaves | 6 | 10 | 24 | 40 | 22 | 101 | 30.2 |
| Veg. sheath | 21 | 18 | 92 | 161 | 54 | 345 | 30.2 | Veg. sheath | 7 | 9 | 32 | 52 | 13 | 113 | 30.0 |
| Veg. stem | 21 | 21 | 118 | 160 | 35 | 355 | 29.9 | Veg. stem | 6 | 5 | 16 | 27 | 6 | 60 | 29.7 |
| Inflorescence | 114 | 149 | 269 | 256 | 60 | 848 | 29.0 | Inflorescence | 76 | 112 | 115 | 132 | 25 | 460 | 28.6 |
| Roots | 3 | 11 | 17 | 24 | 10 | 65 | 29.8 | Roots | 1 | 3 | 6 | 6 | 1 | 17 | 29.4 |
| Site mean | | | | | | 466 | 30.1 | Site mean | | | | | | 132 | 29.7 |

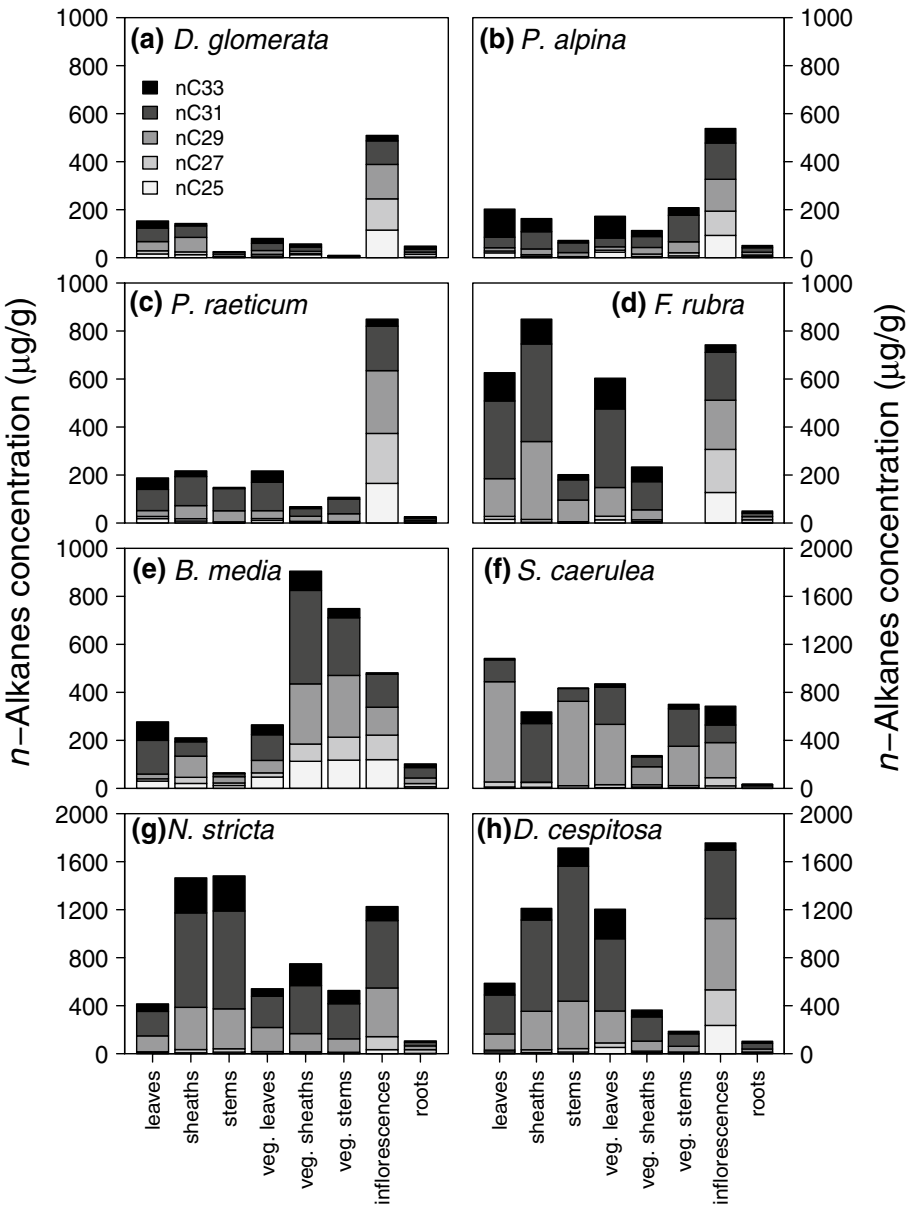


**Fig. 1** *n*-Alkane concentrations (µg/g dry weight) of grass organs at Alp Weissenstein. (Note the change in scale on the *y*-axes for *S. caerulea*, *N. stricta* and *D. cespitosa*)

leaves, sheaths, stems, vegetative green organs deviated from the species mean $\delta^2$H values between −4 and −16 ‰ at Alp Weissenstein and between 3 and −20 ‰ at Ennetbaden, while inflorescences and roots $\delta^2$H values deviated from mean species $\delta^2$H values by 35 and 19 ‰ at Alp Weissenstein and 28 and 14 ‰ at Ennetbaden (Fig. 5).

## Discussion

### *n*-Alkane composition and concentration

We found that carbon autonomous organs (e.g., leaves, sheaths, stems and vegetative tissues) had a higher ACL (>30) than non-autonomous organs (e.g., inflorescences and roots) and that inflorescences had on average the lowest ACL values in a plant (Table 1; Figs. 1, 2). We also found a high across-species variability in ACL with values ranging from 29.3 to 30.5 at Alp Weissenstein and from 27.9 to 30.3 at Ennetbaden. ACL values at Alp Weissenstein were in general higher than at Ennetbaden which could be associated with different environmental conditions at different altitudes. Despite several studies addressing this question, the biological function of ACL is not clear and contradicting results and explanations are found in the literature. For example, Sachse et al. (2006) suggested that longer chain lengths indicated better drought resistance, while Hoffmann et al. (2013) showed variable values. Dodd

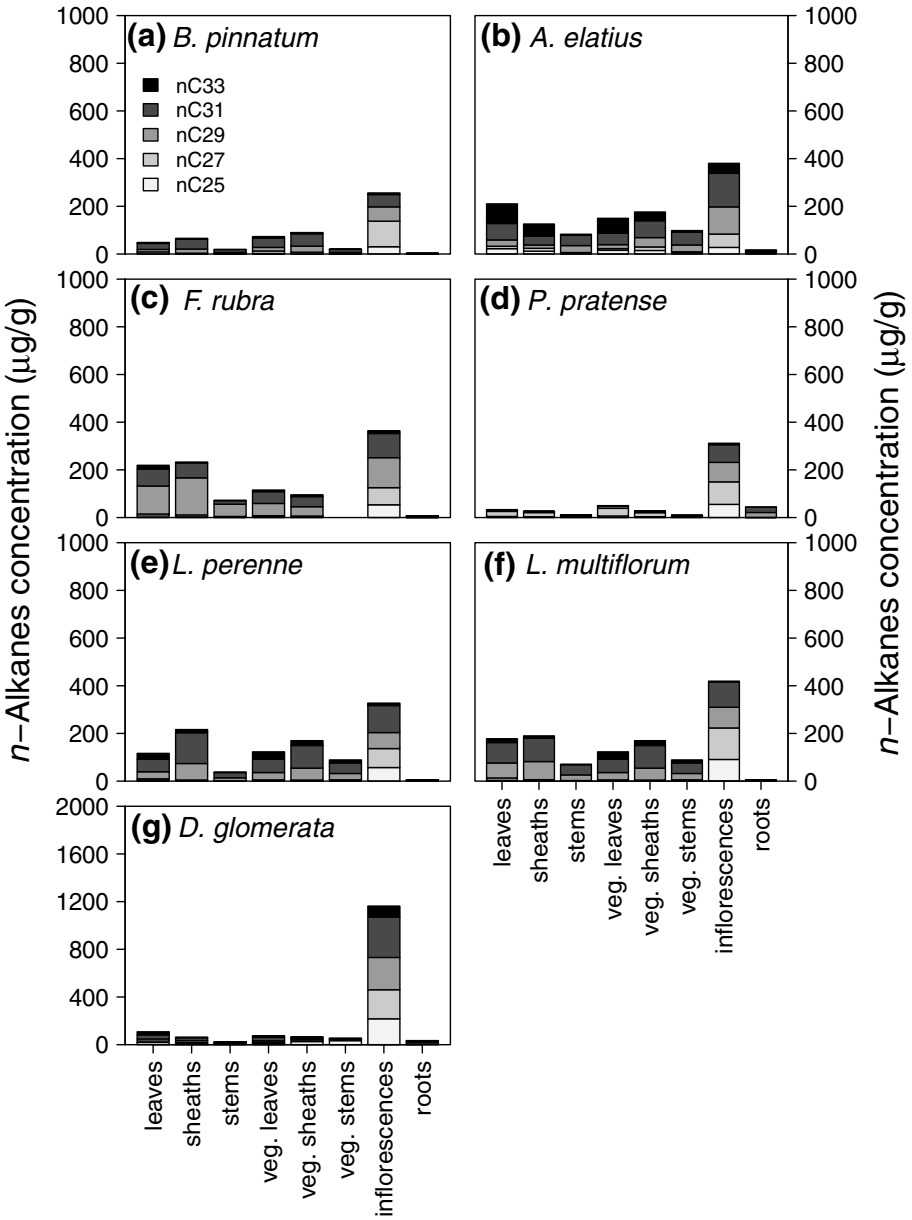


**Fig. 2** *n*-Alkane concentrations (µg/g dry weight) of grass organs at Ennetbaden. (Note the change in scale on the *y*-axes for *D. glomerata*)

**Table 2** Results of an ANOVA testing the effects of species, organs and sites on total *n*-alkane concentrations in grasses from Alp Weissenstein and Ennetbaden

| Factor | *df* | Sum Sq | Mean Sq | *F* value | *p* value |
|---|---|---|---|---|---|
| Species | 11 | 7,611,298 | 691,936 | 10.6822 | <0.001 |
| Organ | 7 | 3,270,726 | 467,247 | 7.2134 | <0.001 |
| Site | 1 | 187,904 | 187,904 | 2.9009 | 0.0917 |
| Residuals | 98 | 6,347,915 | 64,775 | | |

and Poveda (2003) have suggested that summer drought and winter physiological drought could explain greater chain length at low and high altitudes in plant populations from the Pyrenees. In general, our study supports previous findings that indicate an environmental effect on ACL but the biological functions of a changing ACL still remains unclear.

We found that average *n*-alkane concentrations of the individual species (averaged across all organs) ranged from 130 to 890 µg/g at Alp Weissenstein and from 65 to 200 µg/g at Ennetbaden (Figs. 1, 2). These values are in the same range as have been reported for other grassland species (Zhang et al. 2004; Rommerskirchen et al. 2006; Vogts et al. 2009). We observed that concentrations were in general higher at the alpine site of Alp Weissenstein compared to the temperate site of Ennetbaden. These general differences in concentrations between the two sites

**Table 3** Grass organ *n*-alkane $C_{29}$, $C_{31}$ and weighed $C_{29}$–$C_{31}$ $\delta^2H$ values at Alp Weissenstein and Ennetbaden sites

| Alp Weisenstein | $C_{29}$ | $C_{31}$ | $C_{29}$–$C_{31}$ | Ennetbaden | $C_{29}$ | $C_{31}$ | $C_{29}$–$C_{31}$ |
|---|---|---|---|---|---|---|---|
| *D. glomerata* | | | | *B. pinnatum* | | | |
| Leaves | −248 | −244.2 | −245.9 | Leaves | −259.4 | −261.9 | −261.2 |
| Sheath | −243.7 | −253.6 | −248.1 | Sheath | −256.5 | −270.3 | −266.2 |
| Stem | −271.8 | −274.7 | −273.2 | Stem | −273.0 | −283.0 | −279.7 |
| Veg. leaves | −241.0 | −243.6 | −242.7 | Veg. leaves | −270.5 | −271.1 | −270.9 |
| Veg. sheath | n.a. | −278.0 | −278.0 | Veg. sheath | −248.6 | −269.4 | −262.5 |
| Veg. stem | n.a. | n.a. | n.a. | Veg. stem | −271.8 | −287.6 | −282.1 |
| Inflorescence | −214.5 | −223.3 | −218.1 | Inflorescence | −205.3 | −181.0 | −194.1 |
| Roots | −230.1 | −220.9 | −224.8 | Roots | −205.0 | −216.4 | −210.0 |
| Species mean | | | −247.2 | Species mean | | | −253.4 |
| *P. alpina* | | | | *A. elatius* | | | |
| Leaves | | | | Leaves | −259.4 | −225.1 | −222.1 |
| Sheath | −235.0 | −236.0 | −235.8 | Sheath | −256.5 | −229.7 | −229.0 |
| Stem | −245.0 | −245.0 | −245.0 | Stem | −273.0 | −248.6 | −247.3 |
| Veg. leaves | n.a. | n.a. | n.a. | Veg. leaves | −270.5 | −224.9 | −226.1 |
| Veg. sheath | −234.1 | −240.2 | −237.9 | Veg. sheath | −248.6 | −247.1 | −244.8 |
| Veg. stem | n.a. | −252.9 | −252.9 | Veg. stem | −271.8 | −263.2 | −261.8 |
| Inflorescence | −216.8 | −223.6 | −220.4 | Inflorescence | −205.3 | −219.2 | −218.9 |
| Roots | −218.8 | −222.2 | −220.9 | Roots | −205.0 | −217.8 | −213.1 |
| Species mean | | | −235.5 | Species mean | | | −232.9 |
| *P. raeticum* | | | | *D. glomerata* | | | |
| Leaves | −254.2 | −251.2 | −251.8 | Leaves | −223.3 | −225.5 | −224.7 |
| Sheath | −258.7 | −253.1 | −254.8 | Sheath | −244.1 | −250.7 | −248.0 |
| Stem | −285.7 | −283.1 | −284.0 | Stem | −245.8 | −256.2 | −251.7 |
| Veg. leaves | −264.3 | −254.0 | −256.2 | Veg. leaves | −241.0 | −243.6 | −242.7 |
| Veg. sheath | −246.3 | −250.9 | −249.1 | Veg. sheath | n.a. | n.a. | n.a. |
| Veg. stem | −275.4 | −274.0 | −274.5 | Veg. stem | n.a. | n.a. | n.a. |
| Inflorescence | −204.8 | −215.7 | −209.4 | Inflorescence | −204.6 | −209.7 | −207.4 |
| Roots | −238.3 | −238.6 | −238.5 | Roots | −228.4 | −216.6 | −224.6 |
| Species mean | | | −252.3 | Species mean | | | −233.2 |
| *F. rubra* | | | | *F. rubra* | | | |
| Leaves | −256 | −255.8 | −255.8 | Leaves | −247.4 | −243.5 | −245.9 |
| Sheath | −257.8 | −261.8 | −260.0 | Sheath | −233.9 | −227.1 | −232.0 |
| Stem | −262.7 | −263.3 | −263.0 | Stem | −233.5 | −224.8 | −231.5 |
| Veg. leaves | n.a. | −275.2 | −275.2 | Veg. leaves | −240.7 | −240.7 | −240.7 |
| Veg. sheath | −247.2 | −260.4 | −256.9 | Veg. sheath | −235.5 | −224.0 | −229.5 |
| Veg. stem | n.a. | n.a. | n.a. | Veg. stem | n.a. | n.a. | n.a. |
| Inflorescence | −203.5 | −201.3 | −202.4 | Inflorescence | −186.2 | −186.9 | −186.5 |
| Roots | −233.7 | −222.2 | −227.6 | Roots | −218.4 | −201.2 | −214.5 |
| Species mean | | | −248.7 | Species mean | | | −225.8 |
| *N. stricta* | | | | *P. pratense* | | | |
| Leaves | −267 | −267 | −267.2 | Leaves | −183.4 | −169.5 | −180.0 |
| Sheath | −277.6 | −268.0 | −270.9 | Sheath | −182.2 | −167.1 | −178.5 |
| Stem | −257.5 | −265.6 | −263.2 | Stem | −194.2 | −203.6 | −196.6 |
| Veg. leaves | −239.1 | −247.1 | −243.6 | Veg. leaves | −201.8 | n.a. | −201.8 |
| Veg. sheath | −244.9 | −254.4 | −251.8 | Veg. sheath | −222.5 | n.a. | −222.5 |
| Veg. stem | −251.7 | −261.9 | −259.1 | Veg. stem | n.a. | n.a. | n.a. |
| Inflorescence | −240.1 | −256.9 | −249.9 | Inflorescence | −192.3 | −189.5 | −191.0 |
| Roots | −213.0 | −217.4 | −215.2 | Roots | −220.2 | | −220.2 |
| Species mean | | | −252.6 | Species mean | | | −198.6 |

**Table 3** continued

| Alp Weisenstein | $C_{29}$ | $C_{31}$ | $C_{29}$–$C_{31}$ | Ennetbaden | $C_{29}$ | $C_{31}$ | $C_{29}$–$C_{31}$ |
|---|---|---|---|---|---|---|---|
| *B. media* | | | | *L. perenne* | | | |
| Leaves | n.a. | −248 | −248.2 | Leaves | −221.9 | −222.3 | −222.2 |
| Sheath | n.a. | −212.0 | −212.0 | Sheath | −269.6 | −267.5 | −268.2 |
| Stem | −220.7 | n.a. | −220.7 | Stem | −253.2 | −251.0 | −251.8 |
| Veg. leaves | −244.0 | −242.7 | −243.1 | Veg. leaves | −241.6 | −233.8 | −236.6 |
| Veg. sheath | n.a. | −270.4 | −270.4 | Veg. sheath | −242.9 | −245.5 | −244.6 |
| Veg. stem | −223.0 | −235.7 | −229.1 | Veg. stem | −253.8 | −258.2 | −256.5 |
| Inflorescence | −205.1 | −203.3 | −204.1 | Inflorescence | −211.2 | −206.5 | −208.3 |
| Roots | −224.4 | −221.0 | −222.2 | Roots | n.a. | −222.9 | −222.9 |
| Species mean | | | −231.2 | Species mean | | | −238.9 |
| *S. caerulea* | | | | *L. multiforme* | | | |
| Leaves | −252.3 | −230 | −248.3 | Leaves | −248.1 | −239.7 | −243.2 |
| Sheath | −272.0 | n.a. | −272.0 | Sheath | −257.2 | −261.3 | −259.5 |
| Stem | −279.7 | n.a. | −279.7 | Stem | −250.2 | −259.8 | −256.3 |
| Veg. leaves | −261.9 | −241.4 | −254.1 | Veg. leaves | −241.6 | −233.8 | −236.6 |
| Veg. sheath | −242.9 | −223.6 | −236.1 | Veg. sheath | −242.9 | −245.5 | −244.6 |
| Veg. stem | −254.3 | −231.2 | −243.1 | Veg. stem | −253.8 | −258.2 | −256.5 |
| Inflorescence | −200.4 | −220.8 | −207.2 | Inflorescence | −219.6 | −220.7 | −220.2 |
| Roots | −236.4 | −211.7 | −226.6 | Roots | n.a. | −222.9 | −222.9 |
| Species mean | | | −245.9 | Species mean | | | −242.5 |
| *D. cespitosa* | | | | | | | |
| Leaves | −262.1 | −256.1 | −257.8 | | | | |
| Sheath | −297.6 | −292.6 | −294.1 | | | | |
| Stem | −285.6 | −281.2 | −282.3 | | | | |
| Veg. leaves | −296.8 | −282.9 | −287.1 | | | | |
| Veg. sheath | −288.8 | −296.0 | −293.9 | | | | |
| Veg. stem | −273.5 | −277.1 | −276.0 | | | | |
| Inflorescence | −192.6 | −190.7 | −191.7 | | | | |
| Roots | −239.7 | −252.8 | −248.3 | | | | |
| Species mean | | | −266.4 | | | | |
| Alp Weisenstein | | | | Ennetbaden | | | |
| Leaves | −256.7 | −250.4 | −253.6 | Leaves | −228.3 | −226.8 | −228.5 |
| Sheath | −263.2 | −253.9 | −256.0 | Sheath | −238.6 | −239.1 | −240.2 |
| Stem | −263.6 | −268.8 | −263.9 | Stem | −242.2 | −246.7 | −245.0 |
| Veg. leaves | −257.8 | −255.2 | −257.4 | Veg. leaves | −238.2 | −241.3 | −236.5 |
| Veg. sheath | −250.7 | −259.2 | −259.3 | Veg. sheath | −238.8 | −246.3 | −241.4 |
| Veg. stem | −255.6 | −255.5 | −255.8 | Veg. stem | −259.6 | −266.8 | −264.3 |
| Inflorescence | −209.7 | −217.0 | −212.9 | Inflorescence | −205.4 | −201.9 | −203.8 |
| Roots | −229.3 | −225.8 | −228.0 | Roots | −215.6 | −216.3 | −218.3 |
| Site mean | | | −248.3 | Site mean | | | −234.7 |

were consistent among the three species that were sampled at both locations (*D. glomerata, F. rubra* and *P. raeticum*). Our data thus suggest that environmental differences between the two sites rather than the identity of species are causing differences in *n*-alkane concentrations between them. Several previous studies have reported changes in *n*-alkane concentrations along latitudinal environmental gradients (e.g., Hoffmann et al. 2013), where *n*-alkanes tend to increase with increasing aridity. Reports of altitude effects on *n*-alkane concentrations in plants are, however, rare. Salasoo (1989) found a relative *n*-alkane increase with altitude in the leaf cuticular wax composition of *Ericaceae*. However, no absolute amounts were reported in this study. In addition, several previous studies have assessed effects of altitude on cuticle thickness, yet with mixed results. While some studies report increasing cuticle thickness with altitude as a response to increased UV-B radiation (Anfodillo et al. 2002), others have reported reduced cuticle

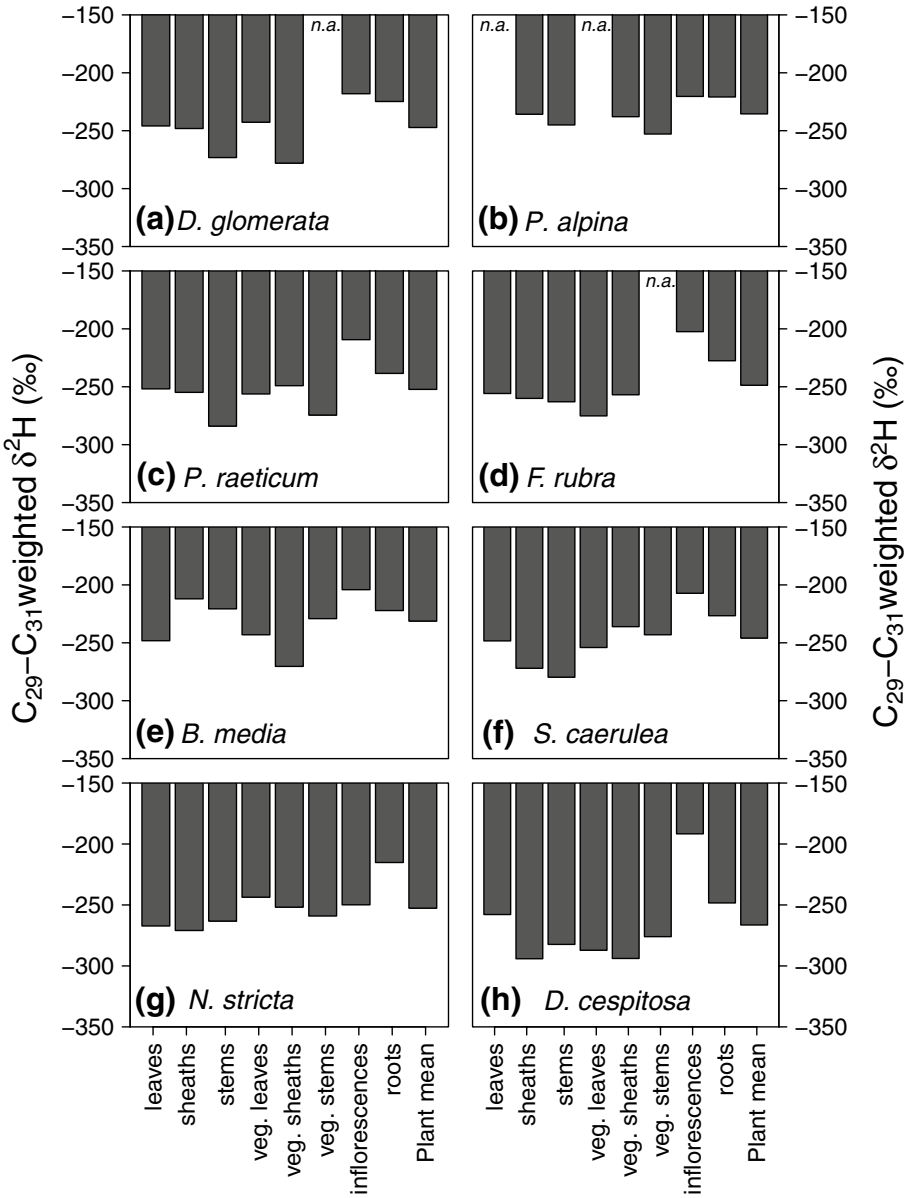


**Fig. 3** Organ $nC_{29}$–$nC_{31}$ weighted *n*-alkane $\delta^2H$ values at Alp Weissenstein. Samples with insufficient *n*-alkane concentrations for hydrogen isotope analyses are marked *n.a.* Note that for each plant organ only a single sample consisting of tissue from >20 bulked individuals was analyzed

thickness (DeLucia and Berlyn 1984; Day et al. 1992). It is, however, unclear whether cuticle thickness and the abundance of *n*-alkanes per gram dry matter correlate in plants. It would thus be interesting to assess in a future study whether the trend of increased *n*-alkane abundance with increasing altitude that we report for grasses can also be found for other taxa in different biomes.

Our study revealed that *n*-alkanes in grasses are not only present in the cuticle of leaf blades but that other parts of the leaf (sheaths) and other plant organs also contain substantial amounts. In particular, inflorescences had a tendency to have the highest *n*-alkane concentrations in most species at both sites, on some occasions exceeding the concentrations of leaves by one order of magnitude (Table 1; Figs. 1, 2). Interestingly, the presence of prevalent $nC_{29}$ and $nC_{31}$ *n*-alkanes in inflorescences is accompanied by overproportionally high concentrations of shorter length alkanes, such as $nC_{25}$ or $nC_{27}$, hence a lower ACL (Table 1). Among others, plants are synthesizing *n*-alkanes and other wax compounds in order to prevent water losses (Jetter et al. 2006; Buschhaus et al. 2007). Inflorescences are the most critical organ in a plant to ensure reproductive success. Since grass inflorescences are wind-pollinated, they typically exceed the grassland canopy and are exposed to the atmosphere above the boundary layer, where evaporative demand is typically higher than within the canopy (Dietrich and Körner 2014; Jones 2014). As such, inflorescences of grasses and possibly also those of

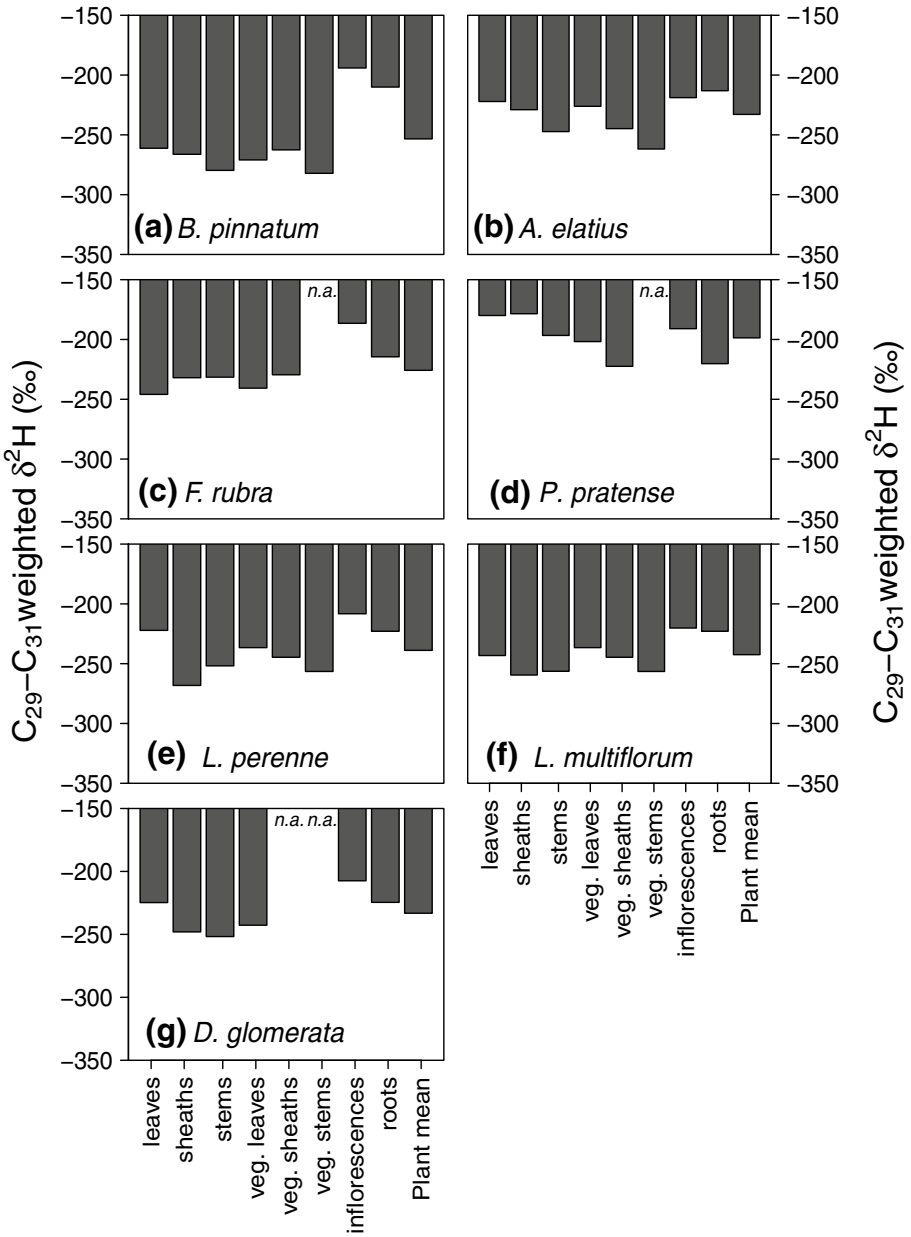


**Fig. 4** Organ $nC_{29}$–$nC_{31}$ weighted *n*-alkane $\delta^2H$ values at Ennetbaden. Samples with insufficient *n*-alkane concentrations for hydrogen isotope analyses are marked *n.a.* Note that for each plant organ only a single sample consisting of tissue from >20 bulked individuals was analyzed

**Table 4** Results of an ANOVA testing the effects of species, organs and sites on *n*-alkane $\delta^2H$ values in grasses from Alp Weissenstein and Ennetbaden

| Factor | *df* | Sum Sq | Mean Sq | *F* value | *p* value |
|---|---|---|---|---|---|
| Species | 11 | 12,260.3 | 1114.6 | 4.2502 | <0.001 |
| Organ | 7 | 29,153.1 | 4164.7 | 15.8813 | <0.001 |
| Site | 1 | 9161.1 | 9161.1 | 34.9338 | <0.001 |
| Organ:site | 7 | 1041.7 | 148.8 | 0.5675 | 0.7803 |
| Residuals | 85 | 22,290.4 | 262.2 | | |

other taxonomic groups are prone to desiccation (Dietrich and Körner 2014). Other than leaves, which are redundant and can be replaced in the course of a season, loss of inflorescences by desiccation would result in loss of sexual offspring, thus a reduced evolutionary fitness. As such, a strong cuticle and high *n*-alkane concentrations might be a strategy of the plant to reduce water permeability and make sure that inflorescences are desiccation resistant, despite being exposed to winds above the canopy boundary layer. In line with our finding, Rommerskirchen et al. (2006)

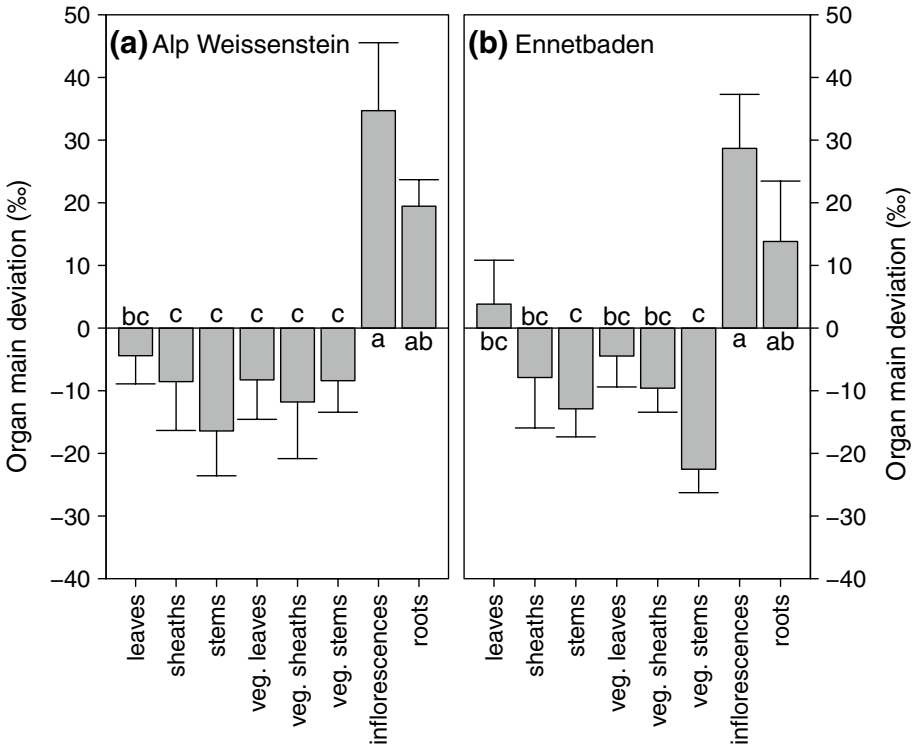


**Fig. 5** Deviation of organ $\delta^2$H values from the plant's mean $\delta^2$H values averaged seperately for Alp Weissenstein (**a**) and Ennetbaden (**b**). $\delta^2$H values of individual plant organs were standardized for each species by calculating the deviation of an organ's $\delta^2$H value from the respective species mean $\delta^2$H value across all organs. These numbers were then averaged across all species at a site. *Letters* indicate significant differences between organs. *Error bars* show one standard deviation from the plant's mean at a location

found that the total *n*-alkane content in flower heads of *Sporobolus* sp. and *Brachiaria* sp., was also 3 and 6 times larger than in leaves and stems.

Opposite to inflorescences, we found that roots had only low amounts of *n*-alkanes (Table 1; Figs. 1, 2). In fact, we found that roots were in general the organs with the lowest *n*-alkane concentrations in all species at both sites. This can be explained because the root tip is meant to be permeable to water to facilitate water uptake by the plant. Here, a water-impermeable waxy layer containing high concentrations of *n*-alkanes would be counter-productive. Also, older roots do not have a cuticle to prevent water loss. Instead, older roots develop a suberized bark to prevent water loss as they mature (Fahn 1990). Given these anatomical and functional differences between root surfaces and other plant organs, the low *n*-alkane concentrations we found for roots are thus not surprising. Our results confirm several previous studies that have also shown very low *n*-alkane concentrations in plant roots when compared to other plant organs. For example, Espelie et al. (1980) studied the composition of cuticular (from aerial organs)- and suberin (from roots)-related waxes of seven plant species. In this study, it was found that the amount of total lipid waxes for roots was very small, and varied from only 10 to 50 μg/g. More recently, Dawson et al. (2000) observed that, in 5 grass species (*L. perenne, P. trivialis, A. capillaris, F. rubra* and *F. ovina*), the average concentration of $nC_{27}$–$nC_{33}$ alkanes in shoots and roots was 187 and 11 μg/g, respectively. It has also been shown that the $nC_{29}$ and $nC_{31}$ alkane concentration from aerial organs was usually higher than those of the underground component (Dawson et al. 2000). Li et al. (2007) investigated the wax biosynthesis and enzymatic relations in wild *Arabidopsis thaliana*. It was found that stems, leaves, siliques, seed and roots had wax loads of ca. 860, 80, 1500, 170 and 360 μg/g, respectively.

Despite our and previous findings, several authors have recently suggested that roots could contribute significant amounts of *n*-alkanes to soils and sediments (Huang et al. 2011; Gocke et al. 2011). If true, this would have important implications for the interpretations of isotope ratios of *n*-alkanes derived from soils or sediments when these are employed in a paleo-environmental context (Sachse et al. 2012). Kuhn et al. (2010), for example, have suggested that leaves and roots from C4 grasses are likely to contribute short-length alkanes to the *n*-alkane pool found in soils. In their study, however, the amount of long-chain *n*-alkanes ($C_{29}$–$C_{31}$) in leaves also surpasses those of the roots. Huang et al. (2011) have also suggested that below-ground plant organs could be an important source of lipids in soils and sediments especially for sterols and ketones. Yet, in this study, only one species out of ten had higher *n*-alkane concentrations in roots than in leaves. Gocke et al. (2011) indicated that roots from different plant species would possibly be a source of *n*-alkanes to soils. However, their direct analysis of lipid concentrations also revealed a higher presence of *n*-alkanes in above-ground tissues. Although these studies have suggested the possibility of root lipid transfer (especially short-length alkanes and other non-alkyl lipids) to soils and sediments, their's and our results clearly indicate that roots are by far the plant organ with the

lowest amount of long-chained alkanes when compared to above-ground organs such as leaves and inflorescences.

### *n*-Alkane $\delta^2H$ values

We found high across-species variability of *n*-alkane $\delta^2H$ values (Tables 3, 4). It is well established that three factors usually determine the *n*-alkane $\delta^2H$ values: source water $\delta^2H$ values, leaf water $^2H$-enrichment, and biosynthetic $^2H$-fractionation (Sachse et al. 2012). As expected, *n*-alkane $\delta^2H$ values at Alp Weissenstein were generally lower than at Ennetbaden. This pattern can mainly be attributed to the source water at high altitudes which has usually more negative $\delta^2H$ values than that of lowerlands (Craig 1961; Siegenthaler and Oeschger 1980).

We also found substantial variability in *n*-alkane $\delta^2H$ values across different organs within a species (Figs. 3, 4). When *n*-alkane $\delta^2H$ values are standardized across species by calculating the deviation of an organ $\delta^2H$ value of a respective species mean, it becomes evident that (except for leaves at the Ennetbaden site) all carbon autonomous organs had more negative $\delta^2H$ values than the plant mean (however, if *P. pratense* was omitted from our analysis, leaves at Ennetbaden would turn from positive to negative values; see Fig. 5). In contrast, non-carbon autonomous plant organs such as inflorescences and roots always had more positive *n*-alkane $\delta^2H$ values than the plant mean at both sites (Fig. 5). The finding that *n*-alkanes derived from non-carbon autonomous plant organs are $^2H$-enriched compared to other plant organs is in line with bulk $\delta^{13}C$ analysis of different plant organs, where heterotrophic organs are also often more enriched than autotrophic organs in $^{13}C$ (Cernusak et al. 2009). Although the drivers of these differences are most likely different for carbon and hydrogen isotopes, it is plausible that in both cases biochemical fractionations are responsible for this effect.

There are two possible drivers that determine differences in the *n*-alkane $\delta^2H$ values among different plant organs. Firstly, leaves and inflorescences might be exposed to microclimates that have an effect on the $^2H$-enrichment of leaf water. Given that inflorescences have fewer stomata (Larcher 2003) and at the same time larger loads of *n*-alkanes than other organs (preventing cuticular transpiration), they are thus unlikely to experience a larger evaporative $^2H$ enrichment compared to other plant organs, in particular when compared to leaves. In addition, roots also show enriched *n*-alkane $\delta^2H$ values compared to other plant organs such as leaves, sheaths and stems. Being located in the soil, roots are, however, not exposed to an environment with high evaporative demand. As such, it is unlikely that evaporative enrichment of root water causes the enriched $\delta^2H$ values of *n*-alkane that we observed in roots of the plants we investigated here. Alternatively, differences in biosynthetic fractionation might cause the observed differences in *n*-alkane $\delta^2H$ values among different plant organs. In general, *n*-alkane hydrogen has three sources: water, acetyl-CoA, and NADPH. Furthermore, NADPH can have two distinct sources of hydrogen. It can come either directly from the light reaction of photosynthesis or from the pentose phosphate pathway where stored carbohydrates are oxidized (Shin 2004; White et al. 2012). NADPH from the light reaction is more depleted in $^2H$ compared to the NADPH from the pentose phosphate pathway of stored carbohydrates (Luo et al. 1991; Schmidt et al. 2003). A likely explanation for our results is, therefore, that *n*-alkanes formed in leaves, stems and sheaths, i.e. in carbon autonomous plant organs, obtained larger amounts of H derived from NADPH from the light reaction and thus show more negative *n*-alkane $\delta^2H$ values. In contrast, the non-green and non-carbon autonomous plant organs, roots and inflorescences, are decoupled in their tissue formation from photosynthesis and obtain more $^2H$-enriched H that comes from NADPH originating in the pentose phosphate pathway. Zhang et al. (2009) have demonstrated that the source of NADPH is an important source of lipid $\delta^2H$ values variability in autotrophically and heterotrophically grown bacteria. In their study, lipids derived from autotrophically growing bacteria were significantly $^2H$-depleted compared to bacteria that had a heterotrophic metabolism. This is directly in line with the patterns we report here, where the green and carbon autonomous organs leaves, sheaths and stems are $^2H$-depleted compared to the non-carbon autonomous plant organs roots and inflorescences. We therefore conclude that different biochemical hydrogen isotope fractionations as a result of organ-specific differences in carbon autonomy and carbon allocation cause the organ-specific differences in *n*-alkane $\delta^2H$ values within a plant.

Interestingly, our findings shed new light on very early analyses of within-plant variability of hydrogen isotopes conducted by Ziegler et al. (1976). Just as we report for *n*-alkanes here, Ziegler et al. (1976) detected that bulk samples from C autonomous plant organs (such as shoots) have more negative $\delta^2H$ values than non-autonomous carbon organs (such as roots). With the new data we report here, and in combination with recent insight into the biochemical hydrogen fractionation processes obtained from bacterial growth cultures (Zhang et al. 2009), it is now possible to suggest the biochemical mechanisms that determine this plant internal variability in $\delta^2H$ values of carbon-autonomous and non-carbon-autonomous plant organs reported nearly 40 years ago.

### Consequences for the interpretation of sediment records

To fully evaluate how *n*-alkanes derived from different plant organs determine the $\delta^2H$ values of the sediment

**Table 5** Estimated contribution of *n*-alkanes from the plant organs shoots (leaves and stems), roots and inflorescences to the sediment record

| Grass organ | Organ biomass in reproducing grasses (%) | Organ biomass in vegetative grasses (%) | Average organ biomass (ecosystem level) (%) | *n*-Alkane concentration (μg/g) | Organ-derived *n*-alkane (μg) per gram of grass | *n*-Alkane contribution to the sediment record (%) |
|---|---|---|---|---|---|---|
| Shoots | 50 | 55 | 54 | 286 | 154.44 | 83 |
| Roots | 40 | 45 | 44 | 41 | 18.04 | 10 |
| Inflorescences | 10 | 0 | 2 | 654 | 13.08 | 7 |

Values for organ-specific biomass allocation are average values estimated from the literature (see text). *n*-Alkane concentrations are across-site averaged values derived from this study

record, mass balance assessments of *n*-alkane production in the individual plant organs are needed. Such assessments are, however, complex because biomass allocation to different plant organs is highly variable across and within species and often controlled by environmental conditions (e.g., Körner 1991). As such, there is no single ratio that can be applied to biomass allocation to different grass organs, and calculating the transfer of organ specific *n*-alkanes to soils and sediments is thus difficult. However, we performed rough estimates of biomass allocation to grass organs based on previously published data in the literature. Depending on the species and environmental variables such as soil moisture, temperature, wind and soil nutrients, the biomass ratio of different organs ranges between 20 and 60 % for shoots (including stems and leaves) and 20–50 % for roots, while inflorescences typically contributed less than 20 % (Potvin 1986; Körner and Renhardt 1987; Rice et al. 1992; Retuerto and Woodward 1992; Kalapos et al. 1996; Rickey and Anderson 2004; Schwinning et al. 2005).

In addition to records of standing biomass, information on biomass turnover of individual organs, i.e. the amount of standing biomass turning into necromass and litter during a species lifetime, is needed to understand plant organ-specific contributions of *n*-alkanes to the sediment record. Such values are, however, even more difficult to obtain than standing biomass, in particular for roots. In fact, root biomass turnover is a key, yet unanswered, question in the global carbon cycle. Data reported in the literature suggest higher biomass and turnovers for leaves than for roots of *Arrhenatherum elatius, Dactylis glomerata* and *Bromus erectus*, especially under high nutrient conditions (Schläpfer and Ryser 1996). In addition, full root turnover in grasses has been estimated to take 4–13 years (Shaver and Billings 1975; Bell and Bliss 1978). Thus, turnover rates for individual grass organs seem to be slower for roots than for grasses but are again highly variable and depend on a number of biological and environmental variables.

We used data on biomass allocation to different organs in grasses that are available from the literature and multiplied these with the organ-specific *n*-alkane concentrations that we report here to roughly estimate which plant organs contribute most to the sediment record (Table 5). In summary, these simplistic estimates show that shoots (leaves and stems), inflorescences and roots contribute on average 83, 7 and 10 %, respectively, of the *n*-alkanes to the sediment record, highlighting that the majority of *n*-alkanes in the sediment record are in fact derived from shoots. Please note, however, that these are very simplistic "back-of-an-envelope" calculations and that relative contributions will vary in nature, depending on species and ecosystem type as well as environmental conditions.

## Conclusions

Our study brings new insights into the causes of natural variability of *n*-alkane $\delta^2H$ values in plants and has implications for the interpretation of *n*-alkanes $\delta^2H$ values in ecological, environmental and paleohydrological research. We show that *n*-alkane concentrations and $^2H$ isotopic composition differ greatly, not only across species and sites but also within plant organs. In contrast to above-ground organs such as leaves, shoots and inflorescences, roots have only low *n*-alkane concentrations. In addition, we found that $\delta^2H$ values are significantly more negative for carbon-autonomous organs such as leaves, sheaths, stems and vegetative organs while non-carbon-autonomous inflorescences and roots have more positve $\delta^2H$ values. We attribute this variability to the carbon metabolism of different plant organs and different associated NADPH sources.

Studies assessing the drivers of $\delta^2H$ variability in *n*-alkanes derived from plants have to date been largely conducted on leaves (Sessions et al. 1999, Sachse et al. 2012). *n*-Alkanes observed in the soil and sediment matrix are, however, a mixture of leaf- and inflorescence-derived lipids. The real contribution of *n*-alkanes from different plant organs to the sediment record is difficult to judge. Based on a simple mass balance calculation, we conclude, however, that leaves are in fact the main source of *n*-alkanes in the sediment. As such, studies assessing the environmental and physiological drivers of *n*-alkanes that focus on leaves produce relationships that can be employed to interpret the $\delta^2H$ values of *n*-alkanes derived from

sediments. This is despite the significant differences that we find among the $\delta^2H$ values in the different plant organs.

**Author contribution statement** A.K. conceived and designed the experiments. A.K. and B.G. conducted the fieldwork. B.G. performed the lab experiments. B.G. analyzed the data. B.G. wrote the manuscript.

**Acknowledgments** B.G. and A.K. were both funded by the ERC starting grant COSIWAX (ERC-2011-StG Grant Agreement No. 279518) to A.K. We thank Nadine Brinkmann and Marc-André Cormier for their help during the fieldwork. We acknowledge Francesca McInerney and one anonymous referee for improving the quality of the manuscript. We thank the Grassland group and the Physiological Plant Ecology group at the Department of Environmental System Sciences at ETH Zurich (D-USYS) for their contribution as host laboratory and for technical support.

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