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Coupling δ^2H and δ^{18}O biomarker results yields information on relative humidity and isotopic composition of precipitation – a climate transect validation study

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Abstract. The hydrogen isotopic composition (δ^2H) of leaf waxes, especially of *n*-alkanes ($\delta^2H_{n-alkanes}$), is increasingly used for paleohydrological and paleoclimate reconstructions. However, it is challenging to disentangle past changes in the isotopic composition of precipitation and changes in evapotranspirative enrichment of leaf water, which are both recorded in leaf wax δ^2H values. In order to overcome this limitation, Zech M. et al. (2013) proposed a coupled $\delta^2H_{n-alkane}$–$\delta^{18}O_{sugar}$ biomarker approach. This coupled approach allows for calculating (i) biomarker-based "reconstructed" δ^2H/δ^{18}O values of leaf water ($\delta^2H/\delta^{18}O_{leaf\ water}$), (ii) biomarker-based reconstructed deuterium excess (*d*-excess) of leaf water, which mainly reflects evapotranspirative enrichment and which can be used to reconstruct relative air humidity (RH) and (iii) biomarker-based reconstructed $\delta^2H/\delta^{18}O_{precipitation}$ values.

Here we present a climate transect validation study by coupling new results from δ^2H analyses of *n*-alkanes and fatty acids in topsoils along a climate transect in Argentina with previously measured δ^{18}O results obtained for plant-derived sugars. Accordingly, both the reconstructed RH and $\delta^2H/\delta^{18}O_{precipitation}$ values correlate highly significantly with actual RH and $\delta^2H/\delta^{18}O_{precipitation}$ values. We conclude that compared to single $\delta^2H_{n-alkane}$ or $\delta^{18}O_{sugar}$ records, the proposed coupled $\delta^2H_{n-alkane}$–$\delta^{18}O_{sugar}$ biomarker approach will allow more robust $\delta^2H/\delta^{18}O_{precipitation}$ reconstructions in future paleoclimate research. Additionally, the proposed coupled $\delta^2H_{n-alkane}$–$\delta^{18}O_{sugar}$ biomarker approach allows for the establishment of a "paleohygrometer", more specifically, the reconstruction of mean summer daytime RH changes/history.

1 Introduction

Long-chain *n*-alkanes and fatty acids are important components of the epicuticular leaf waxes of terrestrial plants (Eglinton and Hamilton, 1967; Samuels et al., 2008). As leaf waxes can be preserved in sedimentary archives over a long time, they serve as valuable biomarkers for paleoen-

vironmental and paleoclimate reconstructions (Eglinton and Eglinton, 2008; Zech M. et al., 2011b). The δ^2H isotopic composition of leaf waxes is of particular interest in this regard because, at least to the first order, it reflects the isotopic composition of precipitation δ^2H_{prec} (Sauer et al., 2001; Huang et al., 2004; Sachse et al., 2004; Schefuss et al., 2005; Pagani et al., 2006; Tierney et al., 2008; Rao et al., 2009), which in turn depends on temperature, the amount of precipitation, atmospheric circulation, etc. (Dansgaard, 1964; Rozanski et al., 1993; Gat, 1996; Araguas-Araguas et al., 2000). While there is probably no fractionation of hydrogen isotopes during water uptake by roots (Ehleringer and Dawson, 1992), several studies have shown that leaf water is enriched in ^{2}H compared to source water or precipitation (Flanagan et al., 1991; Yakir, 1992; Sachse et al., 2006; Smith and Freeman, 2006; Farquhar et al., 2007; Feakins and Sessions, 2010). This ^{2}H enrichment, which is also recorded in leaf waxes (Kahmen et al., 2013a, b), can be explained through evapotranspiration and is mainly controlled by relative air humidity (RH), temperature and the isotopic composition of atmospheric water vapor. Indeed, creating a robust reconstruction of δ^2H_{prec} from soils and sedimentary records increasingly has increasingly turned out to be quite challenging, because it is hitherto difficult to disentangle past changes in δ^2H_{prec} and changes in evapotranspirative enrichment of leaf water (Zech R. et al., 2013; Zech M. et al., 2015).

Compared to compound-specific δ^2H analyses, compound-specific δ^{18}O analyses are by far less adopted by the scientific community as of yet (Hener et al., 1998; Juchelka et al., 1998; Jung et al., 2005, 2007; Greule et al., 2008). However, compound-specific δ^{18}O analyses of hemicellulose-derived sugar biomarkers ($\delta^{18}O_{sugars}$) extracted from plants, soils and sediments, in particular, have been proposed to have large potential, especially in paleoclimate/paleohydrological research (Zech M. and Glaser, 2009; Zech M. et al., 2012b). Similar to leaf waxes, hemicellulose-derived sugars record the isotopic composition of water used for metabolism, i.e., the isotopic composition of precipitation altered by evapotranspirative ^{18}O enrichment of soil and leaf water (Tuthorn et al., 2014; Zech M. et al., 2014a). Recently, Zech M. et al. (2013) proposed a conceptual coupled $\delta^2H_{n-alkane}$-$\delta^{18}O_{sugar}$ model for paleoclimate research and suggested that this coupling allows for overcoming the above defined limitation of single $\delta^2H_{n-alkane}$ approaches. Accordingly, the coupled $\delta^2H_{n-alkane}$–$\delta^{18}O_{sugar}$ approach allows for reconstructing (i) $\delta^2H/\delta^{18}O_{leaf\ water}$ values, (ii) deuterium excess (d-excess) of leaf water, which mainly reflects evapotranspirative enrichment and can be used to reconstruct relative air humidity (RH) and (iii) $\delta^2H/\delta^{18}O_{prec}$ values.

The study presented here aimed at evaluating the coupled $\delta^2H_{n-alkane}$–$\delta^{18}O_{sugar}$ biomarker approach by applying it to a modern topsoil climate transect from Argentina. More specifically, we aimed at (i) analyzing and comparing the δ^2H values of n-alkanes and fatty acids, (ii) modeling ^{2}H leaf water enrichment along the transect and comparing of $\delta^2H_{leaf\ water}$ values with $\delta^2H_{n-alkane}$ and $\delta^2H_{fatty\ acid}$ values, (iii) reconstructing d-excess of leaf water using the coupled $\delta^2H_{n-alkane}$-$\delta^{18}O_{sugar}$ approach and evaluating the potential for reconstructing RH and (iv) reconstructing "biomarker-based" $\delta^2H/\delta^{18}O_{prec}$ values and comparing them with actual $\delta^2H/\delta^{18}O_{prec}$ values.

2 Materials and methods

2.1 Transect description and samples

The investigated transect in Argentina spans ∼ 32 to 47° S, and encompasses 20 sampling locations spanning a large climate and altitudinal (22–964 m) gradient (Fig. 1). Mean annual temperature ranges from 11.4 to 18.0 °C and mean annual precipitation from 185 to 1100 mm (GeoINTA, 2012). Precipitation shows a systematic southward trend towards more negative δ^{18}O and δ^2H values ($\delta^{18}O_{prec}$ and δ^2H_{prec}, respectively; Bowen, 2012).

The transect is described in detail by Tuthorn et al. (2014) and Ruppenthal et al. (2015). Briefly, it is characterized by warm humid subtropical conditions in the north (Zárate, Buenos Aires Province), pronounced arid conditions in the middle part of the transect and cool temperate conditions in the south (Las Heras, Santa Cruz Province). These markedly contrasting climate conditions are reflected in the vegetation zones of the study area, changing from humid/dry Pampas (with dominance of *Triticum, Setaria, Eragrostis, Andropogon, Panicum* and *Festuca* species) in the north to the Espinal vegetation zone (with dominance of *Festuca and Larrea* species) which prevails under semi-arid climate (Burgos and Vidal, 1951), Low Monte semidesert/desert (with dominance of *Larrea* species) in the most arid region of Argentina (Fernández and Busso, 1997) and Patagonian Steppe (with dominance of *Stipa* species) in the southernmost part of the transect (Le Houérou, 1996; Paruelo et al., 1998).

During a field campaign in March and April 2010, mixed topsoil samples (A_h-horizons) from maximum 51 cm depth were collected in triplicate replication from the 20 sample sites along the transect (for soil type and total organic carbon contents please see Table 1 of Tuthorn et al., 2014). The soil samples were air-dried in the field and later in an oven at 50 °C for several days. The sampling site heterogeneity was checked for the $\delta^{18}O_{sugar}$ analyses and in most cases did not exceed the analytical uncertainty (Table 2 in Tuthorn et al., 2014). Therefore, the field replications were merged into one composite sample per study site for the δ^2H_{lipid} analyses.

2.2 Compound-specific δ^2H analyses of n-alkanes and fatty acids

For δ^2H analyses of n-alkane and fatty acid biomarkers, an Accelerated Solvent Extractor (Dionex ASE 200) was used to extract free lipids from the dried soil samples with

Table 1. δ^2H values of individual leaf wax *n*-alkanes and fatty acids. Measurements were carried out in at least triplicate (SD = standard deviation).

	$\delta^2H_{n-alkanes}$				$\delta^2H_{fatty\ acids}$									
sampling locality	C_{29} mean (‰)	SD	C_{31} mean (‰)	SD	C_{22} mean (‰)	SD	C_{24} mean (‰)	SD	C_{26} mean (‰)	SD	C_{28} mean (‰)	SD	C_{30} mean (‰)	SD
1	−157	2	−164	2	−155	1	−157	1	−151	1	−153	1	−153	2
2	−166	0	−166	1	−150	0	−155	1	−165	1	−163	1	−161	3
3	−175	1	−179	1	−162	0	−161	1	−165	1	−159	1	−155	0
4	−176	1	−176	1	−162	2	−163	1	−166	1	−165	1	−158	2
5	−178	1	−180	2	−164	0	−165	1	−168	2	−162	1	−159	1
6	−171	2	−172	0	−166	0	−165	2	−169	1	−161	1	−158	1
7	−179	0	−182	0	−170	0	−172	1	−177	0	−169	1	−157	0
8	−162	1	−167	1	−161	1	−161	1	−166	1	−161	1	−158	2
9	−173	1	−168	1	−163	1	−164	0	−168	1	−169	0	−156	1
10	−173	2	−170	2	−159	1	−167	1	−168	0	−159	1	−137	2
11	−170	2	−156	2	−158	0	−169	0	−167	2	−153	4	−147	4
12	−155	1	−176	0	−158	1	−168	1	−172	1	−148	1	−133	1
13	−157	2	−161	1	−158	1	−153	0	−140	1	−135	1	−128	1
14	−158	1	−166	0	−168	1	−183	0	−181	2	−160	2	−147	1
15	−194	2	−193	1	−194	0	−197	0	−191	1	−176	2	−168	2
16	−203	1	−211	1	−204	1	−198	0	−201	0	−193	0	−189	1
17	−218	1	−217	1	−219	1	−220	1	−217	0	−205	1	−204	1
18	−213	1	−202	4	−211	0	−203	1	−204	0	−196	0	−194	0
19	−222	1	−222	1	−220	0	−210	0	−225	1	−212	1	−204	1
20	−220	1	−212	1	−225	0	−221	1	−211	1	−193	3	−195	2

dichloromethane (DCM) and methanol (MeOH; 9 : 1) according to Zech R. et al. (2013). The total lipid extracts were separated over pipette columns filled with ∼ 2 g aminopropyl. *n*-alkanes were eluted with hexane, more polar lipids with DCM / MeOH (1 : 1) and free fatty acids with diethyl ether / acetic acid (19 : 1). The *n*-alkanes were further purified using zeolite (Geokleen) pipette columns. The zeolite was dried and dissolved in hydrofluoric acid after eluting branched- and cyclo-alkyl compounds with hexane, and the straight-chain (*n*-alkyl) compounds were then recovered by liquid–liquid extraction with hexane. For samples 1–12, an additional purification step with silver nitrate columns was carried out in order to eliminate unsaturated compounds. The chromatograms of the other samples displayed no requirement for this purification step.

Fatty acids were methylated using 5 % HCl in methanol at 80 °C for 12 h. Subsequently, liquid–liquid extraction with 5 % NaCl and hexane was used to retrieve fatty acid methyl esters (FAMEs). FAMEs were purified by elution with dichloromethane over SiO_2 columns (∼ 2 g).

5α androstane and hexamethylbenzene was used for quantification of the compounds using an Agilent Technologies 7890A gas chromatograph (GC) equipped with a VF1 column (30 m, 0.25 mm i.d., 0.25 µm film thickness) and a flame ionization detector (FID). Compound-specific δ^2H values of the long-chain *n*-alkanes and FAMEs were determined based on at least triplicate analyses using a gas chromatograph–pyrolysis–isotope ratio mass spectrometer (GC–pyrolysis–IRMS, Delta V, ThermoFisher Scientific, Bremen, Germany). The A4 standard mixture (provided by Arndt Schimmelmann, Indiana University, USA) was run three times per sequence at three different concentrations. All results are reported after normalization using multi-linear regression (Paul et al., 2007) and simple mass-balance correction of the FAMEs for the isotopic composition of the methanol used for derivatization. Long-term precision of the analyses was monitored using a laboratory standard (oak, *n*-C_{29}). The standard was analyzed in every sequence and yielded a mean value of −147.2 ‰ with a standard deviation of ±1.7 ‰ across all sequences run for this study.

2.3 Modeling of leaf water ^{2}H enrichment

The empirical data analyses were combined with mechanistic model simulations of $\delta^2H_{leaf\ water}$ in order to better detect and evaluate how the dominant climate variables (air temperature and relative air humidity) influence ^{2}H enrichment in lipids. The ^{2}H enrichment of leaf water due to evapotranspiration can be predicted by using mechanistic models originally developed for isotope fractionation processes associated with evaporation from water surfaces by Craig and Gordon (1965). These models were adapted for plants by Dongmann et al. (1974) and subsequently by Flanagan et al. (1991) and Farquhar and Lloyd (1993). Evaporative ^{2}H enrichment of the leaf water (Δ^2H_e) at the evaporative surface in the mesophyll is given by the equation

$$\Delta^2H_e = \varepsilon^+ + \varepsilon_k + \left(\Delta^2H_{WV} - \varepsilon_k\right)\frac{e_a}{e_i}, \quad (1)$$

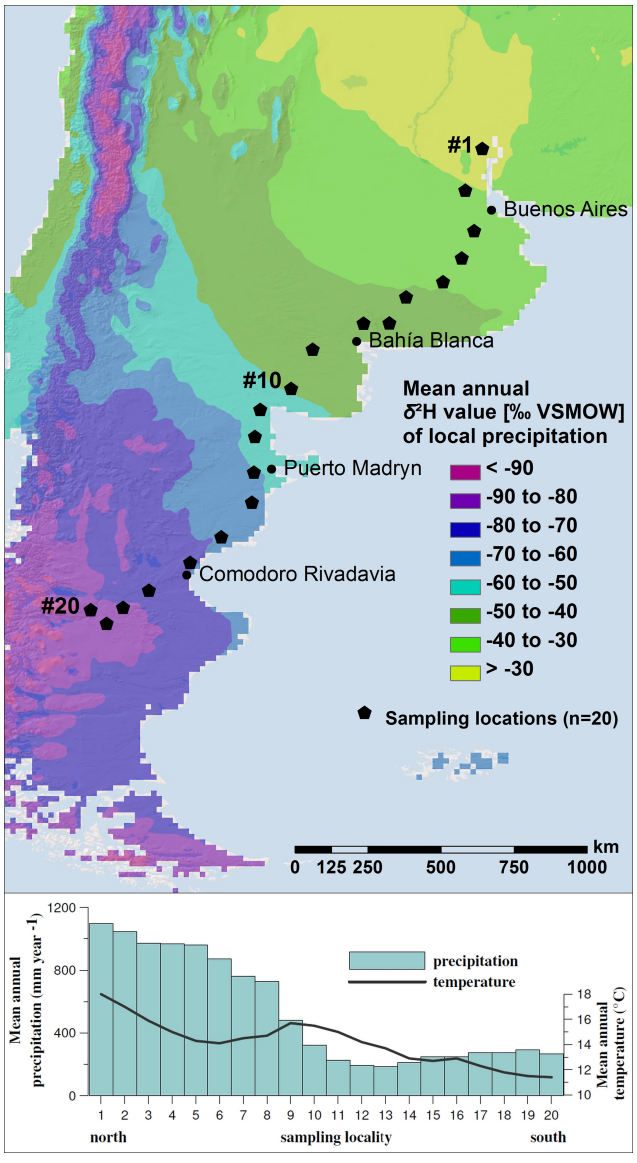

Figure 1. Sampling locations along the investigated transect in Argentina. The colors illustrate the gradient in δ^2H_{prec}, and mean annual temperature and precipitation are shown below.

where ε^+ is the equilibrium fractionation between liquid water and vapor at the air–water interfaces, ε_k is the kinetic fractionation during water vapor diffusion from leaf intercellular air space to the atmosphere, Δ^2H_{WV} is the isotopic difference of the water vapor and the source water and e_a/e_i is the ratio of ambient to intercellular vapor pressure (Farquhar and Lloyd, 1993). This basic calculation was modified by including a Péclet effect that accounts for opposing fluxes of source water entering the leaf through the transpiration flow and the back-diffusion of isotopically enriched water from the sites of evaporation (Farquhar and Lloyd, 1993):

$$\Delta^2H_{\text{leaf water}} = \frac{\Delta^2H_e(1-e^{-\varphi})}{EL/CD}. \quad (2)$$

The quotient of EL/CD represents the Péclet number (φ) where E is the transpiration rate, L is the effective path length, C is the molar concentration of water and D is the diffusivity of $^1H^2HO$. The model approach we used followed that of Kahmen et al. (2011b), where the Péclet-modified Craig–Gordon (PMCG) model is reduced to three input variables: air temperature, atmospheric vapor pressure and source water δ^2H. This simplified model is based on the assumption that throughout the season leaf temperature equals air temperature and that atmospheric vapor δ^2H is generally in equilibrium with source water δ^2H (Kahmen et al., 2011b). Transpiration rates are estimated using relative humidity and air temperature (retrieved from GeoINTA, 2012) and assuming a mean stomatal conductance of 0.15 mol m^{-2} s^{-1}. Based on reports for a large number of species in the literature (Kahmen et al., 2008, 2009; Song et al., 2013), we used an average value of 20 mm for L and kept it constant across the transect. For our simulation of leaf water δ^2H values we obtained the model input variables air temperature and atmospheric vapor pressure from GeoINTA (2012) and source water δ^2H from Bowen (2012), respectively.

The isotopic composition of the leaf water can be estimated according to Eq. (3):

$$\delta^2H_{\text{leaf water}} = \Delta^2H_{\text{leaf water}} + \delta^2H_{SW}, \quad (3)$$

where $\Delta^2H_{\text{leaf water}}$ is the bulk leaf water evaporative enrichment and δ^2H_{SW} is the hydrogen isotope ratio of source / xylem water.

2.4 Conceptual model for the coupled δ^{18}O–δ^2H biomarker approach

The conceptual coupled $\delta^2H_{n-\text{alkane}}$–$\delta^{18}O_{\text{sugar}}$ model was introduced previously by Zech M. et al. (2013). In brief, it is based on the following fundamentals. Precipitation world-wide typically plots along/close to the so-called global meteoric water line (GMWL, $\delta^2H = 8 \times \delta^{18}O + 10$) in a δ^{18}O–δ^2H diagram (Dansgaard, 1964; Fig. 5). Due to fractionation processes, evaporation/transpiration causes water vapor to be isotopically depleted in ^{18}O and ^{2}H, whereas residual (leaf) water ($\delta^2H/\delta^{18}O_{\text{leaf water}}$) is isotopically enriched. In a δ^{18}O–δ^2H diagram, leaf water therefore does not plot on the GMWL but on an evaporation line (EL). The distance of leaf water to the GMWL can be described as deuterium excess ($d = \delta^2H - 8 \times \delta^{18}O$). Using a Craig-Gordon model adapted by Gat and Bowser (1991), the d-excess of leaf water can be used to calculate RH values normalized to the temperature of leaf water (M. Zech et al., 2013):

$$RH = 1 - \frac{\Delta d}{(\varepsilon_2^* - 8 \cdot \varepsilon_{18}^* + C_k^2 - 8 \cdot C_k^{18})}, \quad (4)$$

where Δd represents the difference in d-excess between leaf water and source water. According to Merlivat (1978), experimentally determined kinetic isotope fractionation equals

25.1 and 28.5 ‰ for C_k^2 and C_k^{18}, respectively, considering that these are the maximum values of kinetic fractionation during molecular diffusion of water through stagnant air. Equilibrium isotope enrichments ε_2^* and ε_{18}^* as functions of temperature can be calculated using empirical equations of Horita and Wesolowski (1994). Hence, provided that *n*-alkanes and sugars in plants and soils reflect (albeit with a constant offset caused by biosynthetic fractionation) the isotopic composition of leaf water, a coupled $\delta^2H_{n-alkane}$–$\delta^{18}O_{sugar}$ approach allows for reconstructing RH values. Furthermore, the biomarker-based "reconstructed" $\delta^2H/\delta^{18}O_{leaf\ water}$ values allow reconstructing the isotopic composition of plant source water, which can be considered in an approximation to reflect $\delta^2H/\delta^{18}O_{prec}$ (illustrated as intercepts of the individual ELs with the GMWL in Fig. 5). Assuming a slope of ∼ 2.82 seems reasonable based both on model considerations and on field observations and laboratory experiments (Allison et al., 1985; Walker and Brunel, 1990; Bariac et al., 1994). For further details on modeling coupled δ^{18}O–δ^2H biomarker results, the reader is referred to Zech M. et al. (2013).

3 Results and discussion

3.1 Comparison of $\delta^2H_{n-alkanes}$ and $\delta^2H_{fatty\ acids}$

The C_{29} and C_{31} *n*-alkane homologues were sufficiently abundant in all samples to be measured for their hydrogen isotopic composition. The δ^2H values range from −155 to −222 ‰ and reveal a similar trend between *n*-C_{29} and *n*-C_{31} along the investigated transect (Table 1 and Fig. 2). While the northern and middle part of the transect is characterized by relatively high δ^2H values (∼ −160 ‰), the southern part of the transect is characterized by considerably more negative δ^2H values (∼ −210 ‰).

The δ^2H values of the fatty acids *n*-C_{22}, *n*-C_{24}, *n*-C_{26}, *n*-C_{28} and *n*-C_{30} range from −128 to −225 ‰ (Table 1 and Fig. 2). In general, there is a good overall agreement between the *n*-alkanes and the fatty acids ($R = 0.96$, $p < 0.001$, $n = 20$; for the weighted means), both showing more negative δ^2H values in the southern than in the northern and middle portions of the transect (Table 1, Fig. 2). Interestingly, the longer homologues *n*-C_{28} and *n*-C_{30} are systematically more enriched, by 3 to 43 ‰, compared to the *n*-alkanes. The same was observed by Chikaraishi and Naraoka (2007), reporting on *n*-alkanes being depleted in ^{2}H relative to the corresponding *n*-alkanoic acid. The reasons for this trend remain vague at this point, but may be related to metabolic pathways, seasonal differences in homologue production or differences in homologue sources. Roots, for example, have also been suggested as a source of long-chain *n*-fatty acids (Bull et al., 2000). Shorter homologues, have been suggested to be not only plant-derived, but also of bacterial origin (Matsumoto et al., 2007; Bianchi and Canuel, 2011). Similarly, soil microbial overprinting of long-chain *n*-alkanes and fatty acids cannot be excluded (Nguyen Tu et al., 2011; Zech M. et al., 2011a). By contrast, there is strong evidence suggesting that *n*-alkanes are not significantly introduced into soils/subsoils by roots (Häggi et al., 2014), which seems reasonable given the significantly lower amounts of *n*-alkanes being produced by roots compared to above-ground plant organs (Zech M. et al., 2012a; Gamarra and Kahmen, 2015).

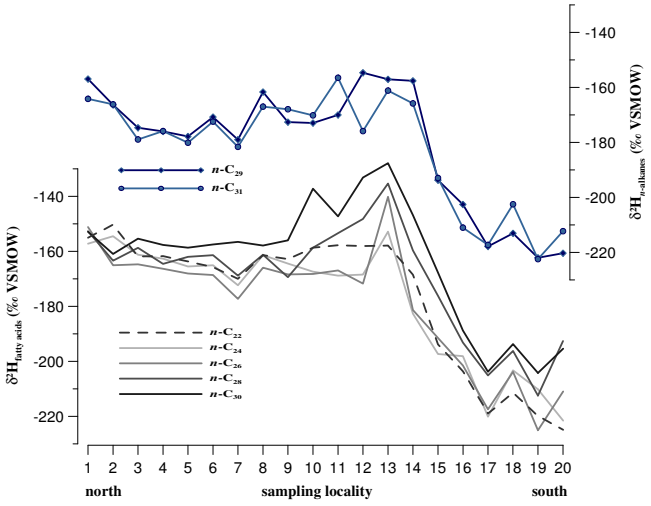

Figure 2. Comparison of δ^2H results of individual leaf wax *n*-alkanes and *n*-alkanoic (fatty) acids along the investigated transect.

The consistent δ^2H pattern revealed by the *n*-alkanes and fatty acids along the north–south climate transect does not solely reflect the δ^2H isotopic composition of precipitation. δ^2H of the lipid biomarkers shows a pronounced offset, especially in the middle part of the transect (Fig. 3). Given that *n*-alkanes are considered to primarily reflect leaf signals and are most widely applied in paleoclimate and paleohydrological studies, we will principally refer to δ^2H of long-chain *n*-alkanes in further discussion and calculations.

3.2 Evapotranspirative ^{2}H enrichment of leaf water

Assuming a constant biosynthetic fractionation of −160 ‰ for the *n*-alkane and fatty acids biosynthesis in plants (Sessions et al., 1999; Sachse et al., 2006), we estimated the isotopic composition of leaf water using our *n*-alkane and fatty acids δ^2H values along the transect/gradient (Fig. 3). Note that an average biosynthetic fractionation factor of ∼ −200 ‰ was reported by Sessions et al. (1999) for short- and mid-chain fatty acids synthesized mostly by unicellular/multicellular marine algae. By contrast, there are hardly any biosynthetic fractionation factors reported for long-chain fatty acids of higher plants. Given that our δ^2H *n*-alkanes and fatty acids values are very similar, using a biosynthetic fractionation factor of −160 ‰ for both lipids seems appropriate.

Estimated leaf water δ^2H values suggest a pronounced greater ^{2}H enrichment of leaf water compared to precipita-

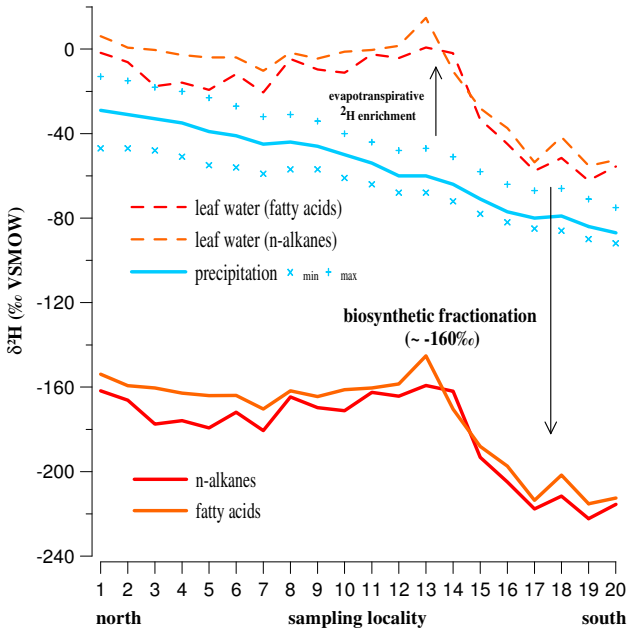

Figure 3. Comparison of measured $\delta^2H_{n\text{-alkanes}}$ (weighted mean of $n-C_{29}$ and $n-C_{31}$) and $\delta^2H_{\text{fatty acids}}$ (weighted mean of $n-C_{22}$, $n-C_{24}$, $n-C_{26}$, $n-C_{28}$ and $n-C_{30}$) patterns with δ^2H_{prec} (Bowen, 2012) along the north–south climate transect (^{x}min and $^{+}$max representing annual minimum and maximum value at the sampling sites). Additionally, assuming a biosynthetic fractionation of −160 ‰ for the *n*-alkane and fatty acid biosynthesis in plants the biomarker-based reconstructed isotopic composition of leaf water is shown.

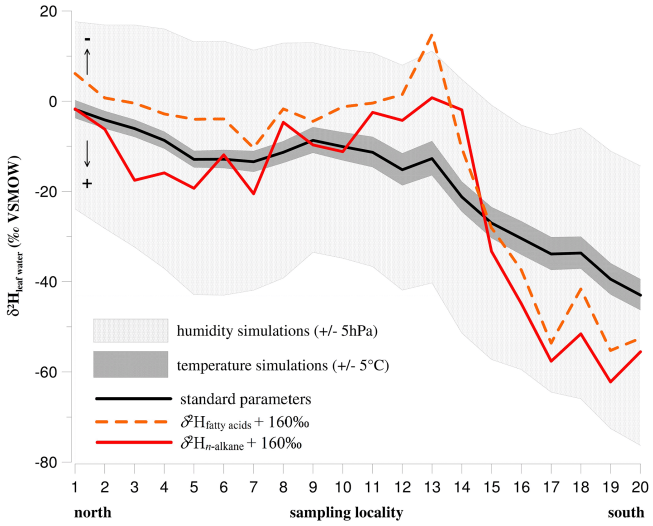

Figure 4. Results of $\delta^2H_{\text{leaf water}}$ model simulations and comparison with biomarker-based reconstructed (assuming a biosynthetic fractionation factor of −160 ‰) isotopic composition of leaf water based on *n*-alkanes and fatty acids, respectively. Sensitivity tests for $\delta^2H_{\text{leaf water}}$ are shown for changes in RH and air temperature for all 20 sites along the transect.

tion (up to +62 ‰). This finding highlights the role of aridity for evapotranspiration and isotopic enrichment of leaf waxes, in good agreement with prior studies (Sachse et al., 2006; Feakins and Sessions, 2010; Douglas et al., 2012; Kahmen et al., 2013a).

Figure 4 illustrates the overall good agreement between $\delta^2H_{\text{leaf water}}$ values inferred from the measured *n*-alkanes and fatty acids, and $\delta^2H_{\text{leaf water}}$ values calculated using the PMCG model. The correlations are highly significant ($r = 0.88$, $p < 0.001$, $n = 20$, for *n*-alkanes and $r = 0.93$, $p < 0.001$, $n = 20$ for fatty acids), suggesting that the model correctly implements the most relevant processes related to evapotranspirative enrichment of leaf water. While predicting the overall trend in leaf water δ^2H along the transect with reasonable accuracy, the model does not capture site-to-site excursions in the *n*-alkane-derived leaf water δ^2H values from this overall trend. As such, additional influences that are not captured by the model, such as possible evaporative ^2H enrichment of soil water (see, e.g., Dubbert et al., 2013), could explain the underestimation of the modeled $\delta^2H_{\text{leaf water}}$ values in the middle part of the transect (Fig. 4). In contrast, the model might overestimate $\delta^2H_{\text{leaf water}}$ in the southern part of the transect. The corresponding ecosystem, the Patagonian Steppe, is a grassland, whereas the middle part of the transect is dominated by shrubland. Grass-derived lipids have been shown to be less strongly affected by evaporative leaf water ^2H enrichment than those of trees or shrubs (McInerney et al., 2011; Yang et al., 2011; Sachse et al., 2012; Kahmen et al., 2013b), and hence the overestimation of the model may be due to plant species effects (Pedentchouk et al., 2008; Douglas et al., 2012). The more pronounced offsets in Patagonia could additionally be attributed to a seasonality effect. The growing season in Patagonia is not year-round but mainly in spring.

In order to assess the sensitivity of the model to the input parameters, we varied vapor pressure of air by ±5 hPa and mean annual temperature by ±5 °C. Changing e_a in Eq. (1) by ±5 hPa corresponds to changes of RH from ca. 94 to 46 % at the beginning of the transect and 89 to 15 % at the end of the transect. While changes in temperature have only negligible effects on the modeled δ^2H isotopic composition of leaf water, changes in RH yield difference in $\delta^2H_{\text{leaf water}}$ of up to ∼ 30 ‰ (Fig. 4). Different climatic conditions during the spring growing season in Patagonia could thus explain the overestimation of the evapotranspirative enrichment in the model.

Evapotranspirative enrichment of leaf water has also been observed in $\delta^{18}O$ values of hemicellulose-derived arabinose, fucose and xylose analyzed in topsoils along the investigated transect (Tuthorn et al., 2014). Model sensitivity tests of ^{18}O enrichment of leaf water using the PMCG model corroborate the observations presented here that air humidity is the key factor defining the ^{18}O–^2H enrichment of leaf water.

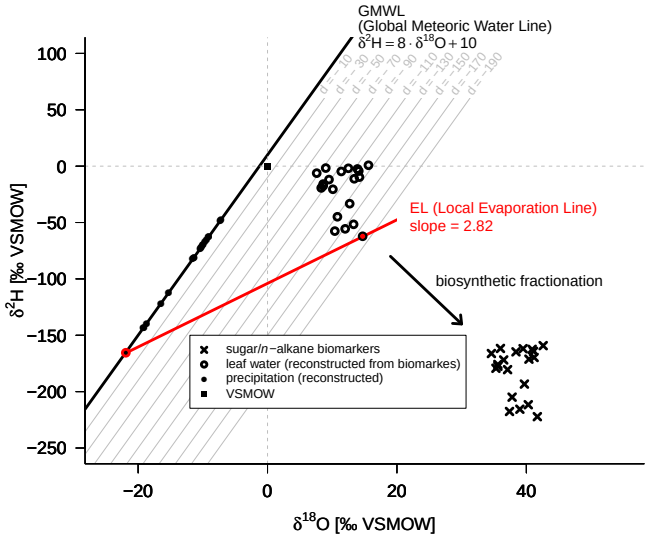

Figure 5. δ^{18}O–δ^2H diagram illustrating the conceptual model of the coupled $\delta^2H_{n-alkane}$-$\delta^{18}O_{sugar}$ approach (modified after Zech M. et al., 2013). $\delta^2H_{n-alkane}$ (mean of n-C_{29} and n-C_{31}) and $\delta^{18}O_{sugar}$ (mean of arabinose, fucose and xylose) results are used to reconstruct $\delta^2H/\delta^{18}O_{leaf\ water}$ by subtracting the biosynthetic fractionation factors. The deuterium excess ($d = \delta^2H - 8{\cdot}\delta^{18}O$) of leaf water serves as proxy for RH, and $\delta^2H/\delta^{18}O_{prec}$ is calculated as the intersection of the individual evaporation lines (ELs, slope of 2.82) with the GMWL.

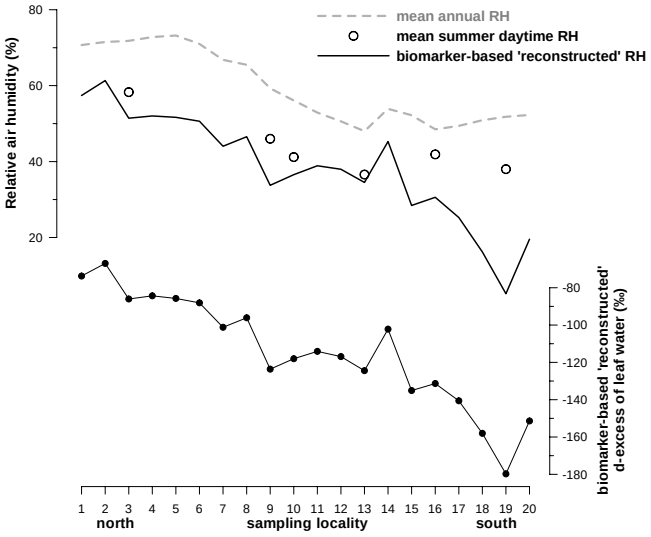

Figure 6. Comparison of biomarker-based reconstructed relative humidity (RH) values with actual RH values (mean annual RH retrieved for all investigated sites from GeoINTA, 2012; mean summer daytime RH for six stations retrieved from www.ncdc.noaa.gov). Deuterium excess values were calculated using $\delta^{18}O_{leaf\ water}$ reconstructed from terrestrial sugars (Tuthorn et al., 2014) and $\delta^2H_{leaf\ water}$ reconstructed from n-alkanes.

3.3 Coupling of the $\delta^2H_{n-alkane}$ and $\delta^{18}O_{sugar}$ biomarker results

The conceptual model for the coupled $\delta^2H_{n-alkane}$–$\delta^{18}O_{sugar}$ biomarker approach is illustrated in Fig. 5. The model is based on the assumption that the investigated n-alkane and hemicellulose biomarkers are primarily leaf-derived and reflect the isotopic composition of leaf water. With regard to the topsoil transect investigated here, this assumption is reasonable and supported by leaf water modeling (for δ^2H in Sect. 3.2, and for δ^{18}O see Tuthorn et al., 2014). Accordingly, biomarker-based reconstructed $\delta^2H/\delta^{18}O_{leaf\ water}$ values can be calculated from the biomarkers by applying biosynthetic fractionation factors ε_{bio}. For our reconstructions, we applied ε_{bio} factors of −160 ‰ (Sessions et al., 1999; Sachse et al., 2006) and +27 ‰ (Sternberg et al., 1986; Yakir and DeNiro, 1990; Schmidt et al., 2001; Cernusak et al., 2003; Gessler et al., 2009) for δ^2H and δ^{18}O, respectively (Fig. 5).

3.3.1 Reconstructed RH values along the climate transect and comparison with actual RH values

The reconstructed d-excess values of leaf water along the investigated transect range from −67 to −178 ‰ and reveal a systematic trend towards more negative values in the south (Fig. 6). The reconstructed RH values calculated using the leaf water d-excess values according to the above-described coupled $\delta^2H_{n-alkane}$–$\delta^{18}O_{sugar}$ approach range from 16 to 65 %, with one extremely low value of 5 % (Fig. 6). Reconstructed RH values follow the systematic d-excess trend and correlate highly significantly ($r = 0.79$, $p < 0.001$, $n = 20$) with the actual mean annual RH values retrieved from GeoINTA (2012) for all investigated sites.

However, as depicted by Fig. 6, the reconstructed RH values systematically underestimate the actual mean annual RH values. This is especially pronounced for the three southernmost locations (18–20) and may be attributed to several causes. First, the applied model calculations do not account for evaporative enrichment of soil water. In the δ^{18}O-δ^2H diagram, the soil water enrichment shifts the source water (simplified to "reconstructed precipitation" in Fig. 5 and our model) along the evaporation line and thus leads to too-negative d-excess values and an underestimation of RH. Second, given that leaf waxes are considered to be formed mostly during early stages of leaf ontogeny (Kolattukudy, 1970; Riederer and Markstaedter, 1996; Kahmen et al., 2011a; Tipple et al., 2013), they may not necessarily reflect the mean annual isotopic composition of precipitation in regions with pronounced seasonality, but rather the isotopic composition of precipitation during the growing season. Furthermore, mean annual RH values likely overestimate the RH values actually seen in photosynthetically active leaves. Indeed, when comparing the biomarker-based reconstructed RH values with mean summer daytime RH values (available for six stations along the investigated transect from www.ncdc.noaa.gov), satisfactory agreement between recon-

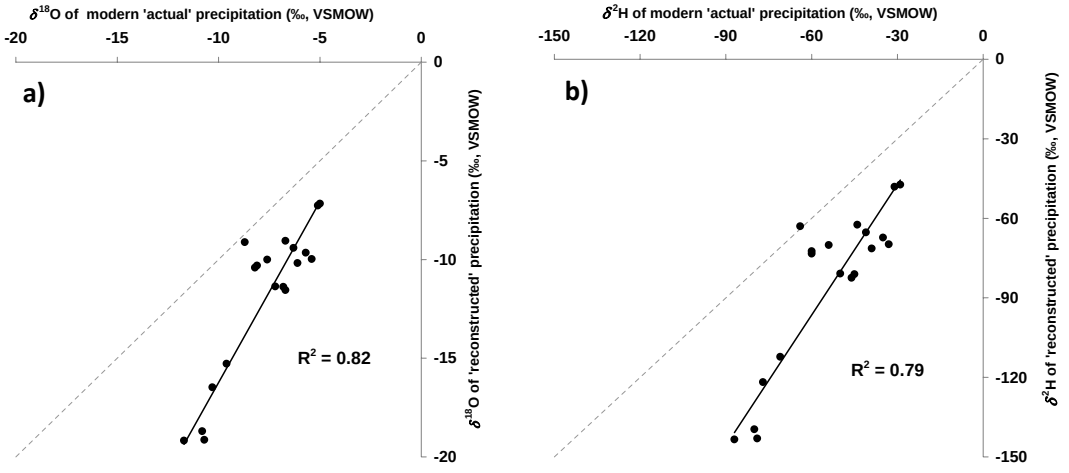

Figure 7. Correlation of biomarker-based reconstructed $\delta^{18}O_{prec}$ and δ^2H_{prec} values with modern "actual" $\delta^{18}O_{prec}$ and δ^2H_{prec} values (from Bowen, 2012).

structed and actual RH values is obtained, with the exception of the southern portion of the transect (Fig. 6). Third, the δ^{18}O biosynthetic fractionation factor of $\sim+27$ ‰, which has been reported for newly assimilated sugars and cellulose, underestimates in our opinion the actual fractionation factor of hemicellulose sugars (Tuthorn et al., 2014; Zech M. et al., 2014a). This results in reconstructed leaf water values plotting too far to the right in the δ^{18}O–δ^2H diagram (Fig. 5), and in turn leading to the observed underestimated RH values (Fig. 6). We argue with the loss of a relatively ^{18}O-depleted oxygen atom attached to C-6 during pentose biosynthesis (C-6 decarboxylation; Altermatt and Neish, 1956; Harper and Bar-Peled, 2002; Burget et al., 2003) and point to a recent study of Waterhouse et al. (2013), who determined the position-specific δ^{18}O values in cellulose. Further experimental studies, like those suggested and encouraged by Sternberg (2014) and Zech M. et al. (2014b), are urgently needed to determine an improved biosynthetic fractionation factor for hemicellulose-derived sugars.

3.3.2 Comparison of reconstructed and actual δ^2H_{prec} and $\delta^{18}O_{prec}$ values

Values of $\delta^{18}O_{prec}$ and δ^2H_{prec} reconstructed as the intercepts of the individual evaporation lines (EL) with the GMWL in the δ^{18}O–δ^2H diagram (Fig. 5) range from -7 to -22 ‰ and from -47 to -166 ‰, respectively. They correlate highly significantly (Fig. 7; $r = 0.90$, $p < 0.001$, $n = 20$, and $r = 0.88$, $p < 0.001$, $n = 20$ for $\delta^{18}O_{prec}$ and δ^2H_{prec}, respectively) with the "actual" [1] δ^2H_{prec} and $\delta^{18}O_{prec}$ values as derived from Bowen (2012). While the reconstructed $\delta^{18}O_{prec}$ and δ^2H_{prec} values, like the reconstructed RH values, generally validate the conceptual coupled δ^{18}O–δ^2H approach, they appear to systematically underestimate the actual δ^{18}O and δ^2H values of the precipitation water (Fig. 7).

The uncertainties discussed above for the observed offset of reconstructed versus actual RH values can also affect the accuracy of reconstructed $\delta^{18}O_{prec}$ and δ^2H_{prec} values. Hence, the actual $\delta^2H/\delta^{18}O_{prec}$ values used for our comparison with the biomarker-based reconstructed values can be assumed to be one of the possible sources of uncertainty. While Bowen (2012) reported a confidence interval (95 %) ranging from 0.2 to 1.2 ‰ and from 2 to 11 ‰ for δ^2H_{prec} and $\delta^{18}O_{prec}$, respectively, future climate transect studies that will be carried out with actual precipitation being sampled for δ^2H–δ^{18}O analyses are encouraged. Moreover, we would also like to emphasize here the very likely influence of seasonality. As reported for sugar biomarkers (Tuthorn et al., 2014), we also suggest that leaf waxes mainly reflect the humidity and the isotopic composition of spring and summer precipitation rather than mean annual values.

4 Conclusions

The hydrogen isotopic composition of leaf wax *n*-alkanes and *n*-alkanoic (fatty) acids extracted from topsoils along a transect in Argentina varies significantly, with δ^2H values ranging from -155 to -222 ‰ and -128 to -225 ‰, respectively. These δ^2H values broadly parallel variations in the hydrogen isotopic composition of precipitation, but are modulated by evaporative ^{2}H enrichment of leaf water. A mechanistic leaf water model correctly simulates the overall trends. Sensitivity tests show that relative humidity exerts

[1] Please note that we chose here the term "actual" for reasons of simplification in order to show the difference from the biomarker-based reconstructed $\delta^{18}O_{prec}$ and δ^2H_{prec} values. Indeed, both the reconstructed and the actual values are derived from modeling, namely from our conceptual $\delta^2H_{n-alkane}$-$\delta^{18}O_{sugar}$ model and from the Bowen (2012) online isotopes in the precipitation calculator.

a much stronger influence on evaporative enrichment than temperature.

In order to evaluate the conceptual coupled $\delta^2H_{n-alkane}$–$\delta^{18}O_{sugar}$ approach proposed by Zech M. et al. (2013), we interpreted the biomarkers extracted from our Argentinean climate transect as such:

- Assuming that the *n*-alkanes and hemicellulose-derived sugars are primarily leaf-derived, we reconstructed $\delta^2H_{leaf\ water}$ and $\delta^{18}O_{leaf\ water}$.
- This in turn allowed us assessing the *d*-excess of leaf water. The large calculated range in *d*-excess along the transect (−67 to −178 ‰) can be used to calculate biomarker-based reconstructed RH values. Reconstructed RH values correlate highly significantly with actual mean annual RH values along the transect. Despite this highly significant correlation, reconstructed RH values systematically underestimate actual mean annual RH values. However, this discrepancy is largely reduced when reconstructed RH values are compared with actual mean summer daytime RH values.
- Similarly, biomarker-based reconstructed $\delta^{18}O_{prec}$ and δ^2H_{prec} values correlate highly significantly with actual $\delta^{18}O_{prec}$ and δ^2H_{prec} values, but reveal systematic offsets, too.

We conclude that compared to single $\delta^2H_{n-alkane}$ or $\delta^{18}O_{sugar}$ records, the proposed coupled $\delta^2H_{n-alkane}$–$\delta^{18}O_{sugar}$ approach will allow more robust $\delta^2H/\delta^{18}O_{prec}$ reconstructions in future paleoclimate studies. Additionally, it allows for establishing a "paleohygrometer", more specifically the reconstruction of mean summer daytime RH changes/history using *d*-excess of leaf water as proxy. However, further studies are needed to determine an improved biosynthetic fractionation factor for hemicellulose-derived sugars. Also, in the light of strong diurnal variations of δ^2H and $\delta^{18}O$ of leaf water, it is important to determine which portion of this diurnal signal is actually incorporated in the *n*-alkanes and sugars being synthesized in the leaves.

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