Dear Editor Prof. Dr. Kuzyakov,
Dear Yakov,

first of all, we would like to thank you very much for your editorial handling of our MS (MS No.: bg-2014-532) entitled:

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

(formerly “Coupled isotopes of plant wax and hemicellulose markers record information on relative humidity and isotopic composition of precipitation”)


Please note that we did not only change the title during revision, but would also be very happy if you could agree that we include Prof. Dr. K. Rozanski as further co-author. He contributed significantly not only to the originally developed coupled $\delta^2$H and $\delta^{18}$O biomarker approach, but also to our MS during this revision.

We are also very grateful to Anonymous Referee#1 and to Referee#2 Marie Galeron for their efforts and constructive comments on our MS.

As you will see from our attached document “revised manuscript with track changes” with invested quite a lot in order to further improve our MS and clarity/readability for our readers (and reviewers). For instance, we clarify already in the title that this is a “climate transect validation study”, we considerably rewrote the abstract as well as the objectives (in the introduction chapter), included “mean summer daytime relative humidity values in revised Fig. 6 and the text, etc…

In the following we address and clarify all questions/issues raised by the referees (lines refer to the original MS):
Reply to Anonymous Referee#1

We thank Anonymous Referee#1 for her/his constructive comments and suggestions on our manuscript, which help to improve the MS and allow us to expand the discussion on some aspects.

Referee: “Coupled isotopes of plant wax and hemicellulose markers record information on relative humidity and isotopic composition of precipitation”, The Authors of present manuscript conducted a major revision of the manuscript. In general the manuscript has been improved. The whole manuscript has been revised accordingly to reviewer’s suggestion and is now more comprehensible. However, some other comments the authors have not addressed too much, especially samples and soil characters. There are still some comments and critical points that should be considered.

Abstract: I also wonder the last sentence (conclusion), which is too long and unclear. Just “support to the coupled $\delta^{2}H_{lipid}$ and $\delta^{18}O_{sugar}$ biomarker approach”, what are the new observations? What are the improvements on the well-known approach? So, I still suggest the authors put forward a clearer conclusion according to the new observations.

Response: Following the recommendation of Reviewer#1, we rewrote the abstract in order to improve readability. Amongst others, we now state explicitly that “compared to single $\delta^{2}H_{n-alkane}$ or $\delta^{18}O_{sugar}$ records, the proposed coupled $\delta^{2}H_{n-alkane}$-$\delta^{18}O_{sugar}$ approach will allow a more robust reconstruction of $\delta^{2}H/\delta^{18}O_{precipitation}$ and additionally the reconstruction of RH changes/history in future paleoclimate research.”

Introduction: The section is informative, and the objective is now clearer.

Method: The depth and some general physic-chemical characteristics of topsoil should be included. Why “The soil samples were air-dried in the field and later in an oven at 50 oC”? How long the time of air-dried and over-dried? Some references are needed. “: : :the field replications were merged to one composite: : :.”, so there are no replicates. I suggest the authors give the readers more convinced reasons.

Response: We now included/refer our readers to Tuthorn et al., 2014: “(for soil type and total organic carbon contents please see Table 1 of Tuthorn et al., 2014)” and included that drying of the samples was carried out for several days. We are not aware of respective references for drying soil samples.
Furthermore, we now explain in more detail that “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 to one composite sample per study site for $\delta^{2}H_{lipid}$ analyses.”

“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 sampled displayed no requirement for this purification step.” Why?? What are the differences among samples?

Response: According to our knowledge certain microorganisms produce unsaturated hydrocarbons. However, given that we are not aware of any respective systematic studies for soils, we cannot answer this question of Reviewer#1.
I still wonder that there are only 20 samples; I don’t think it is correct to calculate them to a general model. Please explain it more clearly.

Response: Please note that the coupled $\delta^{2}H_{n}$-alkane-$\delta^{18}$O$_{\text{sugar}}$ model is not based on the 20 samples analysed in this study for $\delta^{2}H_{n}$-alkanes as assumed by Reviewer#1 (and Reviewer#2). Indeed this is a conceptual model (see title of the respective subchapter 2.4) and for further details on the model we refer our readers to Zech et al. 2013 at the end of the chapter. The 20 samples from the presented Argentinean climate transect are rather used for validating the conceptual coupled $\delta^{2}H_{n}$-alkane-$\delta^{18}$O$_{\text{sugar}}$ model.

Following the request of Reviewer#1 and in order to make this more clear, we (i) slightly changed the title and included amongst others “– a climate transect validation study”, (ii) restructured and reformulated the abstract, (iii) reformulated introduction chapter and the aims of our study and (iv) partly rewrote the conclusion chapter.

Results and discussion: There are sufficient data, figures and tables. I suggest the authors depart these two parts to make the change tendencies and their exploitations much clearer. In the present status, much more observations had not well explained.

Response: Given that all three current subchapters of the Result and Discussion chapter contain discussion aspects, we prefer to maintain the current structure of our MS.

Reply to Referee#2 Marie Galeron

We thank Marie Galeron for her constructive and detailed comments and suggestions on our manuscript. In the following we address all issues raised in her review.

Referee#2: My main concern with this manuscript is the small number of samples used to build the conceptual model. While I understand that the model can be drafted with such few samples, it should be made clear in the manuscript that the conceptual model is not a tool ready for a research use at this stage. The bias observed between the model outputs and the actual modern RH values/$^2$H leaf enrichment could be a concern since the model is not solidly built on a large number of observations.

Response: Please note that the coupled $\delta^{2}H_{n}$-alkane-$\delta^{18}$O$_{\text{sugar}}$ model is not based on the 20 samples analysed in this study for $\delta^{2}H_{n}$-alkanes as assumed by both Reviewer#1 and Marie Galeron. Indeed this is a conceptual model (see title of the respective subchapter 2.4) and for further details on the model we refer our readers to Zech et al. 2013 at the end of the chapter. The 20 samples from the presented Argentinean climate transect are rather used for validating the conceptual coupled $\delta^{2}H_{n}$-alkane-$\delta^{18}$O$_{\text{sugar}}$ model.

In order to make this more clear, we (i) slightly changed the title and included amongst others “– a climate transect validation study”, (ii) restructured and reformulated the abstract, (iii) reformulated the aims of our study at the end of the introduction chapter and (iv) slightly rewrote the conclusion chapter.

Referee#2: Another concern is the assumption that the studied n-alkanes and hemicellulose markers are leaf-derived. I agree that these compounds tend to be tracers of terrestrial higher plants, and more precisely leaves, but there is very little description of the actual vegetation found in sample sites along the transect. There seems to be quite a variation in climate and vegetation across sites, and while I can agree that in the tropical humid areas, leaves will rapidly feed the topsoil layer sampled, without further description, the more arid areas, with hardly any leafy vegetation, could have a different
Profile. Please provide details on species/types of vegetation encountered at sampling sites.

Response: Following the recommendation of Marie Galeron, we included the dominant plant genus in the Material and Method section. Furthermore, we refer our readers to Tuthorn et al. (2014) and now additionally also to Ruppenthal et al. (2015), where maps illustrate the distribution of vegetation zones in the study area and to several references focussing on vegetational research in Argentina. Concerning the leaf-origin of the investigated biomarkers, we agree with Marie Galeron that this is one of the major uncertainties for our conceptual coupled $\delta^{2}H_{n}$-alkane-$\delta^{18}O_{sugar}$ model. We therefore openly discuss the option of stem, root and soil microbial contributions in our MS and complemented the discussion during revision by including that “...there is strong evidence suggesting that $n$-alkanes are not significantly introduced into soils/subsoils by roots (Häggi et al., 2014).”

Referee#2: The source of fatty acids could be numerous (discussed on p. 2468, lines 18-23) – maybe some sampling locations deserve an estimation of leaf-derived vs. non-leaf-derived material?

Response: While we agree with Marie Galeron that a quantitative estimation of leaf-derived versus non-leaf-derived fatty acids would be desirable, we consider this aim to be very ambitious and hardly possible based on the available data.

Referee#2: The Global Meteoric Water Line (concept and uses) should be defined in the manuscript.

Response: Included and rewritten at the beginning of chapter 2.4. Conceptual model for a coupled $\delta^{18}O-\delta^{2}H$ biomarker approach

Referee#2: “Based on the premise that $n$-alkanes and hemicellulose biomarkers are primarily leaf-derived, we reconstruct $\delta^{2}H$leaf water and $\delta^{18}O$leaf water, respectively, which in turn allows assessment of the $d$ excess of leaf water. The large calculated range in $d$ excess along the transect (-67 to -178 ‰) can be used to calculate/model RH.” The leaf water reconstructions, in turn, feed the RH reconstruction model. But RH is needed to estimate transpiration rates used in the leaf water reconstruction model? Isn’t that an issue when coupling the models?

Response: Please allow us to clarify that two models were used in our study. First, (chapter 2.3. and 3.2.) we used a Péclet-modified Craig Gordon model (Kahmen et al., 2011) in order to (i) estimate leaf water enrichment, (ii) support the notion that RH is the main controlling climatic factor and (iii) $n$-alkanes and fatty acids of the topsoils reflect the isotopic composition of precipitation modified by leaf water enrichment. Second, the conceptual coupled $\delta^{2}H_{n}$-alkane-$\delta^{18}O_{sugar}$ model (chapter 2.4. and 3.3.). Here, we reconstruct biomarker-based $\delta^{2}H/\delta^{18}O_{leaf\ water}$ values by correcting for biosynthetic fractionation factors. The reconstructed biomarker-based $\delta^{2}H/\delta^{18}O_{leaf\ water}$ values are then used for calculating RH values.

Referee#2: P. 2472, lines 13-28. This paragraph is confusing. Line 25 “However, give that this...”: what does “this” refer to?

Response: Paragraph rewritten and simplified (partly deleted)
Referee#2: Figure 3: The caption/legend could be clearer.

Response: Following the recommendation of Marie Galeron we added: “Comparison of measured $\delta^2$H$_{n}$-alkanes (weighted mean of n-C$_{29}$ and n-C$_{31}$) and $\delta^2$H$_{\text{fatty acids}}$ (weighted mean of n-C$_{22}$, n-C$_{24}$, n-C$_{26}$, n-C$_{28}$ and n-C$_{30}$) pattern with $\delta^2$H$_{\text{prec}}$ (Bowen, 2012) along the north-south climate transect ($\delta^{\text{min}}$ and $\delta^{\text{max}}$ representing annual minimum and maximum value at the sampling site).”

Referee#2: Figure 5: May be too complicated. Evaporation Lines and GMWL needs to be clearly defined in the text. The legend is not clear as to what it is exactly that is represented. What is the main message that this figure should convey?

Response: In order to make the message of this figure clearer, we rewrote the caption. It now reads: “Fig. 5: $\delta^{18}$O-$\delta^2$H diagram illustrating the conceptual model of the coupled $\delta^{2}$H$_{n}$-alkane-$\delta^{18}$O$_{\text{sugar}}$ approach (modified after Zech M. et al., 2013a). $\delta^{2}$H$_{n}$-alkane (mean of n-C$_{29}$ and n-C$_{31}$) and $\delta^{18}$O$_{\text{sugar}}$ (mean of arabinose, fucose and xylose) results are used to reconstruct $\delta^2$H/$\delta^{18}$O$_{\text{leaf water}}$ by subtracting the biosynthetic fractionation factors. The deuterium excess ($d = \delta^2$H – 8 x $\delta^{18}$O) of leaf water serves as proxy for RH and $\delta^2$H/$\delta^{18}$O$_{\text{prec}}$ is calculated as intersection of the individual evaporation lines (ELs, slope 2.82) with the global meteoric water line (GMWL).”

Furthermore, we changed the symbols (both in the graph and the legend) and rewrote chapter 2.4. Conceptual model for a coupled $\delta^{2}$H-$\delta^{18}$O biomarker approach.

Referee#2: Figure 7: Is this figure necessary?

Response: Yes, it illustrates one of the main results of our MS, namely the highly significant correlation of modern ‘actual’ $\delta^2$H/$\delta^{18}$O$_{\text{prec}}$ with biomarker-based ‘reconstructed’ $\delta^2$H/$\delta^{18}$O$_{\text{prec}}$.

Referee#2: There are a number of issues with the reference list:

- Should the last Zech et al. reference in the list (2013) be 2013c? Please update in the manuscript as well.
- Cited in the text but missing from the reference list:
  - Gessler et al., 2009 (P. 2472, line 25)
  - Kahmen et al., 2009 (P. 2466, line 20)
  - Song et al., 2013 (P. 2466, line 20)
  - In the manuscript, please remove b from Kahmen et al., 2011b reference (P. 2466, line 13, and P. 2472, line 3)
  - P. 2464, line 17: Zech et al., 2013 $\to$ a, b, or c?
  - P. 2472, line 3: Tippel et al. $\to$ 2012 in the manuscript, 2013 in the reference list. Please fix date in the manuscript.

Response: Thanks a lot for pointing us to these flaws $\to$ all corrected

Referee#2: Typos:

- Please make sure the n in n-alkanes is in italics throughout the text
- P. 2462, line 26: “enrichment of leaf water being recorded in both, n-alkanes and...”: please
remove comma
- P. 2463, line 19: “sampling localities”: should this be sampling locations?
- P. 2464, line 25-26: “The chromatograms of the other sampled...”: should this read “The chromatograms of the other sampled...”?
- P. 2465, line 20: space missing between “The” and “2H”
- P. 2469, line 28: please add comma after “enrichment of soil water”, and replace “can possibly” by “could”
- P. 2472, line 1: “Third, given that leaf waxes considered to be...” : Should this read “Third, given that leaf waxes ARE considered to be...”?

Response: Thanks a lot for pointing us to these flaws ➔ all corrected

We hope we could address all comments/questions/suggestions of the referees appropriately and we would be happy to acknowledge both you for your editorial handling and the referees in the acknowledgements.

With kind regards,

Mario Tuthorn & Michael Zech & Co-authors

Attachment: revised manuscript with track changes
Coupled $\delta^2$H and $\delta^{18}$O isotopes of plant wax and hemicellulose markers as biomarkers result in yields information on relative humidity and isotopic composition of precipitation – a climate transect validation study.


* Corresponding author (michael_zech@gmx.de)
Abstract

The δ²H isotopic composition of leaf waxes, especially of n-alkanes (δ²Hₙ-alkanes), is used increasingly for paleohydrological and -climate reconstructions. However, it is challenging to disentangle past changes in the isotopic composition of precipitation and changes in evapotranspirative enrichment of leaf water. In order to overcome this limitation, Zech M. et al. (2013, Chemical Geology 360-361, pp. 220-230) proposed a coupled δ²Hₙ-alkane-δ¹⁸O₅ugar biomarker approach. This coupling allows us to analyze δ²H on n-alkanes and fatty acids in topsoils along a climate transect in Argentina, for which we had previously measured δ¹⁸O on plant-derived sugars. Our results indicate that leaf wax biomarker δ²H values (δ²Hₕₚₐₗₜ) primarily reflect δ²Hₑₚₑₑₑₑ (precipitation), but are modulated by evapotranspirative enrichment. A mechanistic model is able to produce the main trends in δ²Hₕₚₐₗₜ along the transect, but seems to slightly underestimate evapotranspirative enrichment in arid regions and overestimate it in grass-dominated ecosystems. Furthermore, the (i) coupling of the δ²Hₕₚₐₗₜ and δ¹⁸O₅ugar biomarker results and (ii) application of biosynthetic fractionation factors allows calculating biomarker-based reconstructed δ²Hₑₚₑₑₑₑ/δ¹⁸O₅ugar values, isotopic composition of leaf water along the transect. This also yields (i) the deuterium excess (d-excess) of leaf water, which mainly reflects evapotranspirative enrichment, and can be used to model-reconstruct relative air humidity (RH) and (iii) δ²H/δ¹⁸O₅ precipitation values. Here we present a respective climate transect validation study by coupling new results from δ²H analyses on n-alkanes and fatty acids in topsoils along a climate transect in Argentina with previously measured δ¹⁸O results obtained for plant-derived sugars. Accordingly, both the Correlating the reconstructed RH and δ²H/δ¹⁸O₅ precipitation values correlate highly significantly with actual RH and δ²H/δ¹⁸O₅ precipitation values. We conclude that compared to single δ²Hₙ-alkane or δ¹⁸O₅ugar records, the proposed coupled δ²Hₙ-alkane-δ¹⁸O₅ugar approach will allow more robust to the measured RH, as well as the reconstructed and actual isotopic composition of
precipitation, we tested the value of combined $\delta^2$H and $\delta^{18}$O approach for the reconstruction of $\delta^2$H/$\delta^{18}$O$_{precipitation}$, the isotopic signal of past precipitation reconstructions and additionally the reconstruction of RH changes/history in future. We conclude that the good agreement and high correlation between the values lend support to using the combined $\delta^2$H and $\delta^{18}$O measurement of lipid and sugar biomarkers for paleoclimate research. The high correlation of modeled (reconstructed based on biomarker results) and measured RH, as well as the good agreement between modeled and actual $\delta^2$H and $\delta^{18}$O of precipitation along the transect lends support to the coupled $\delta^2$H$_{lipid}$ and $\delta^{18}$O$_{sugar}$ biomarker approach for future paleoclimate research.

Keywords: paleoclimate proxies, hemicellulose sugars, $n$-alkanes, leaf water enrichment, deuterium-excess, relative air humidity
1. Introduction

Long chain n-alkanes and fatty acids are important components of the epicuticular leaf waxes of terrestrial plants (Eglinton, 1967; Samuels et al., 2008). As leaf waxes can be preserved in sedimentary archives over a long time (Radke et al., 2005; Samuels et al., 2008), they can serve as valuable biomarkers for paleo-environmental and -climate reconstructions (Eglinton and Eglinton, 2008; Zech et al., 2011b). The δ²H isotopic composition of leaf waxes is of particular interest in this regard, because, at least to a first order, it reflects the isotopic composition of precipitation δ²H_{precip} (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, humidity, 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 the roots (Ehleringer and Dawson, 1992), several studies have shown that leaf water is enriched in ²H compared to the source water or precipitation (Flanagan et al., 1991; Yakir, 1992; Sachse et al., 2006; Smith & Freeman, 2006; Farquhar et al., 2007; Feakins & Sessions, 2010). This ²H enrichment, which is also recorded in the leaf waxes, can be explained by evapotranspiration and is mainly controlled by relative air humidity (RH), temperature and the isotopic composition of atmospheric water vapor. Indeed, potential variations in the degree of evapotranspirative enrichment in the past can make it challenging to reconstruct the isotopic composition of paleoprecipitation from δ²H biomarker records alone. Compound-specific δ²H of lipid biomarkers, especially n-alkanes due to their good preservation, are already widely applied in paleoclimatic and hydrological research (Sauer et al., 2001; Schefuss et al., 2005; Pagani et al., 2006; Tierney et al., 2008; Zech et al., 2011b). A robust reconstruction of δ²H_{precip} from soils and sedimentary records turns increasingly out to be quite challenging, because it is hitherto difficult to disentangle past changes in δ²H_{precip} and
Changes in evapotranspirative enrichment of leaf water (Zech, R. et al., 2013; Zech, M. et al., 2015). Comparison of $\delta^2$H$_{n$-alkanes} with $\delta^2$H$_{alky}$ in such research is hardly done so far but may provide additional merits of using fatty acids as an alternative to $n$-alkanes. Similarly, compared to compound-specific $\delta^2$H analyses, compound-specific $\delta^{18}$O analyses of sugars remain in their infancy, yet hold significant promise by far less adopted by the scientific community, so far (Henner et al., 1998; Juchelka et al., 1998; Werner, 2003; Jung et al., 2005; Jung et al., 2007; Greule et al., 2008). However, particularly compound-specific $\delta^{18}$O analyses of hemicellulose-derived sugar biomarkers ($\delta^{18}$O$_{sugars}$) extracted from plants, soils and sediments are proposed to have large potential especially in paleoclimate-hydrologic research (Zech M. & Glaser, 2009; Zech M. et al., 2012). 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 (Zech et al., 2013b; Tuthorn et al., 2014; Zech M. et al., 2014a). Hemicellulose residues can persist in soils (Zech et al., 2012). Recently, Zech M. et al. (2013) proposed a conceptual coupled $\delta^2$H$_{n$-alkanes}$-\delta^{18}$O$_{sugars}$ model for paleoclimate research and suggested that this coupling allows overcoming the above defined limitation of single $\delta^2$H$_{n$-alkanes}$ approaches. Accordingly, the coupled $\delta^2$H$_{n$-alkanes}$-\delta^{18}$O$_{sugars}$ approach allows reconstructing (i) $\delta^2$H/$\delta^{18}$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) $\delta^2$H/$\delta^{18}$O$_{prec}$ values. Given the evaporative $^2$H and $^{18}$O enrichment of leaf water being recorded in both, $n$-alkanes and hemicellulose-derived sugars, the combined plant wax $\delta^2$H and hemicellulose sugar $\delta^{18}$O analyses offer the potential of reconstructing the deuterium excess of leaf water. The d-excess quantifies the isotopic deviation of water from the Global Meteoric Water Line (GMWL) and may serve as valuable proxy for evapotranspirative enrichment and RH. Furthermore, if leaf water values are defined in a $\delta^2$H/$\delta^{18}$O diagram...
sugar and n-alkane isotopic information may enable reconstruction of the isotopic composition of precipitation in paleohydrological studies (Zech et al., 2013a).

This here presented study describes the application of a combined approach at validating or falsifying the coupled δ²H and δ¹⁸O biomarker approach by applying it to a modern topsoil sampled along a climate transect from Argentina. The aims of this investigation are to:

(i) analyse and comparing the δ²H values of n-alkanes with those of fatty acids, (ii) modelling n-alkane leaf water enrichment along the transect and comparison with δ²H concentrations and δ²Hfatty acids values and δ¹⁸O values of sugars and evaluate the dominant climate factors influencing these values,

(ii) reconstructing d-excess of leaf water using the coupled δ²Hn-alkane values of n-alkanes and δ¹⁸O values of sugars approach and assess evaluating the potential of reconstructing d-excess of leaf water as paleoclimate proxy for RH, and

(iii) reconstructing ‘biomarker-based’ δ²H/δ¹⁸Oprec values and evaluate the potential of the combined δ²H and δ¹⁸O biomarker approach to reconstruct the comparison with actual δ²H/δ¹⁸Oprec values in isotopic composition of precipitation.

2. Material and methods

2.1. Transect description and samples

The investigated transect in Argentina spans from ~32°S to 47°S, and encompasses 20 sampling localities spanning a large climate and altitudinal (22–964 m) gradient (Fig. 1). Mean annual temperature ranges from 11.4 °C to 18.0 °C and mean annual precipitation from 185 mm to 1100 mm (GeoINTA, 2012). Precipitation shows a systematic southward trend towards more negative δ¹⁸O and δ²H values (δ¹⁸Oprec and δ²Hprec, respectively) (Bowen, 2012; GeoINTA, 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 Pampa (with dominance of *Triticum*, *Setaria*, *Eragrostis*, *Andopogon*, *Panicum* and *Festuca* species) in the north to the Espinal vegetation zone (with dominance of *Festuga* and *Larrea* species) that 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. While the sampling site heterogeneity was checked for the $\delta^{18}O_{\text{sugar}}$ analyses and in most cases did not exceed the analytical uncertainty (Table 2 in Tuthorn et al., 2014), therefore, the field replications were merged to one composite sample per study site for $\delta^2H_{\text{lipid}}$ analyses.

### 2.2. Compound-specific $\delta^2H$ analyses of $n$-alkanes and fatty acids

For $\delta^2H$ 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 dichloromethane (DCM) and methanol (MeOH; 9:1) according to Zech R. et al. (2013a). 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 HF 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 hours. 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).

5\& alpha; androstane and hexamethylbenzene was used for quantification of the compounds on an Agilent Technologies 7890A gas chromatograph (GC) equipped with a VF1 column (30 m, 0.25 mm i.d., 0.25 \( \mu \)m film thickness) and a flame ionization detector (FID). Compound-specific \( \delta^2 \)H values of the long-chain \( n \)-alkanes and FAMEs were determined based on at least triplicate analyses on 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 derivatisation. 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 $\delta^2$H$_{leaf\ water}$ 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 Farquhar and Lloyd (1993). Evaporative $^2$H enrichment of the leaf water ($\Delta^2$H$_L$) at the evaporative surface in the mesophyll is given by the equation:

$$\Delta^2H_L = \varepsilon^+ + \varepsilon_k + (\Delta^2H_{wv} - \varepsilon_k) \frac{\varepsilon^+}{\varepsilon_k},$$

(Eqn. 1)

where $\varepsilon^+$ is the equilibrium fractionation between liquid water and vapor at the air-water interfaces (Bottinga and Craig, 1969), $\varepsilon_k$ is the kinetic fractionation during water vapor diffusion from leaf intercellular air space to the atmosphere, $\Delta^2H_{wv}$ 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 (Craig and Gordon, 1965). 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):

$$\Delta^2H_{leaf\ water} = \frac{\Delta^2H_L}{EL/CD}.$$

(Eqn. 2)

The quotient of EL/CD represents the Péclet number ($\varphi$) 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 $^2$H$_2$O. The model approach we used followed that of Kahmen et al. (2011b), where the Péclet-modified Craig Gordon model is reduced to three input variables: air temperature, atmospheric vapour pressure and source water $^2$H. This simplified model is based on the assumption that leaf temperature equals air temperature and that atmospheric vapor $^2$H is in
equilibrium with source water $\delta^2$H (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/m²/s. Based on reports for a large number of species in the literature (Kahmen et al., 2008; Kahmen et al., 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 $\delta^2$H values we obtained the model input variables air temperature, atmospheric vapor pressure and source water $\delta^2$H from GeoINTA (2012) and Bowen (2012), respectively.

The isotopic composition of the leaf water can be estimated according to Eqn. 3:

$$\delta^2H_{\text{leaf water}} = \Delta^2H_{\text{leaf water}} + \delta^2H_{\text{sw}}$$  (Eqn. 3)

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

### 2.4. The conceptual model for a coupled $\delta^{18}$O-$\delta^2$H biomarker approach

The conceptual coupled $\delta^2H_{\text{alkane}}, \delta^{18}O_{\text{sugar}}, \delta^{18}O_{\text{residual}}$ model was introduced previously by Zech M. et al. (2013). In brief, it is based on the following fundamentals. Precipitation worldwide typically plots along/close to the so-called global meteoric water line (GMWL, $\delta^2H = 8 \times \delta^{18}O + 10$) in a $\delta^{18}O$-$\delta^2H$ diagram (Dansgaard, 1964). (Fig. 5). Due to fractionation processes, evaporation/transpiration causes evaporating water vapour 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 $\delta^{18}$O-$\delta^2$H diagram. Leaf water therefore does not plot on the GMWL but on an evaporation line (EL). The distance of reconstructed leaf water to the Global Meteoric Water Line (GMWL) can be described as the deuterium excess ($d = \delta^2H - 8 \delta^{18}O$). Observed deviation is caused by the kinetic effect resulting in slower diffusivity of the $^2$H$^4$H$^1$O molecules compared to the $^4$H$^4$H$^1$O molecules. More humid conditions and less evapotranspiration are reflected by
lower d values, and more arid conditions and more evapotranspiration are reflected by higher d values. 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 (Zech et al., 2013a):

\[
RH = 1 - \frac{\Delta d}{\left[ \varepsilon_2' - 8 \cdot \varepsilon_1' + C_2' - 8 \cdot C_1' \right]}
\]

(Eqn. 4)

where \(\Delta d\) 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 \(C_2\) and \(C_{18}\), respectively, considering that these are the maximum values of kinetic fractionation during molecular diffusion of water through stagnant air.

Equilibrium isotope enrichments \(\varepsilon_2^*\) and \(\varepsilon_{18}^*\) 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 \(\delta^2H_{\text{alkane}}-\delta^{18}O_{\text{sugar}}\) approach allows reconstructing RH values.

The combined \(\delta^{18}O-\delta^2H\) biomarker The biomarker-based reconstructed \(\delta^2H/\delta^{18}O_{\text{leaf water}}\) values approach also allows furthermore reconstructing of the isotopic composition of plant source water, which can be considered as-in an approximation to reflect \(\delta^2H/\text{prec}\) and \(\delta^{18}O_{\text{prec}}\). Illustrated in Figure 5 these are given by the intercepts of the individual evaporation lines (ELs) with the GMWL. Assuming a slope of ~2.82 seems reasonable both based on model considerations and based on field observations and laboratory experiments. The slope value of 2.82 that is used for the EL has been observed in previous experiments on evaporating leaf water (Allison et al., 1985; Walker and Brunel, 1990; Bariac et al., 1994). For further details on modelling coupled \(\delta^{18}O-\delta^2H\) biomarker results the reader is referred to Zech M. et al. (2013a).
3. Results and Discussion

3.1. Comparison of δ²H-n-alkanes and δ²H-fatty acids

The C₂₉ and C₃₁ n-alkane homologues were sufficiently abundant in all samples to be measured for their hydrogen isotopic composition. The δ²H values range from -155 to -222 ‰ and reveal a similar trend between n-C₂₉ and n-C₃₁ along the investigated transect (Table 1 and Fig. 2). While the northern and middle part of the transect is characterized by relatively high δ²H values (~ -160 ‰), the southern part of the transect is characterized by considerably more negative δ²H values (~ -210 ‰).

The δ²H values of the fatty acids n-C₂₂, n-C₂₄, n-C₂₆, n-C₂₈ and n-C₃₀ 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 much more negative δ²H values in the south than in the northern and middle portions of the transect (Table 1, Fig. 2). Interestingly, the longer homologues n-C₂₈ and n-C₃₀ are systematically 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 ²H relative to the corresponding n-alkanoic acid. Reasons for this trend remain vague at this point, but may be relate 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 produced by plants (Gamarra and
Kahmen, 2015) and not significantly introduced into soils/subsoils by roots (Häggi et al., 2014).

The consistent δ²H pattern revealed by the n-alkanes and fatty acids along the north-south climate transect does not solely reflect the δ²H isotopic composition of precipitation. Especially in the middle part of the transect, δ²H of the lipid biomarkers shows a pronounced offset (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 δ²H of long chain n-alkanes in further discussion and calculations.

3.2. Evapotranspirative δ²H enrichment of leaf water

Assuming a consistent 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 δ²H 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-chained fatty acids synthesized mostly by unicellular/multicellular marine algae. By contrast, there are hardly any biosynthetic fractionation factors reported for long-chained fatty acids of higher plants. Given that our δ²H n-alkanes and fatty acids values are very similar, using a biosynthetic fractionation factor of -160 ‰ for both lipids seems appropriate.

Estimated leaf water δ²H values suggest a pronounced δ²H enrichment of leaf water compared to precipitation (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 δ²H_leaf water values inferred from the measured n-alkanes and fatty acids, and δ²H_leaf water values calculated using the Peclet-
modified Graig-Gordon 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 $\delta^2$H along the transect with reasonable accuracy, the model does not capture site-to-site excursions in the $n$-alkane-derived leaf water $\delta^2$H values from this overall trend. As such, additional influences that are not captured by the model, such as evaporative $^2$H enrichment of soil water, can possibly explain the underestimation of the modeled $\delta^2$H leaf water values in the middle part of the transect (Fig. 4). In contrast, the model might overestimate $\delta^2$H leaf water in the northern and the southern part of the transect. The corresponding ecosystems, the Humid Pampa and the Patagonian Steppe, respectively, are grasslands, 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 $^2$H 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. While changes in temperature have only negligible effects on the modeled $\delta^2$H isotopic composition of leaf water, changes in RH yield difference of up to $\sim30$‰ (Fig. 4). Different climatic conditions during the spring growing season in Patagonia could thus readily 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 analysed in topsoils along the investigated transect (Tuthorn et al., 2014). Model sensitivity tests of $^{18}$O enrichment of leaf water using...
PMCG model corroborate the observations presented here that air humidity is the key factor defining the $^{18}$O/$^2$H enrichment of leaf water.

3.3. Combining Coupling of the $\delta^2$H$_{n}$-alkane and $\delta^{18}$O$_{sugar}$ and $\delta^2$H$_{n}$-alkane-biomarker analysis results

A conceptual model for the combined interpretation of coupled $\delta^2$H$_{n}$-alkane and $\delta^{18}$O$_{sugar}$ biomarker approaches can be illustrated in a $\delta^2$H/$^2$H diagram (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 $\delta^2$H in Section 3.2, and for $\delta^{18}$O see Tuthorn et al., 2014). Accordingly, the isotopic composition of leaf water can be biomarker-based ‘reconstructed’, $\delta^2$H/$^2$H/leaf water values can be calculated from the isotopic composition of the biomarkers by applying biosynthetic fractionation factors $\varepsilon_{\text{bio}}$, an average value according to various studies of the biosynthetic fractionation factors resulting in. For our reconstructions we applied $\varepsilon_{\text{bio}}$ 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 $\delta^2$H and $\delta^{18}$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 Craig-Gordon coupled $\delta^2$H$_{n}$-alkane-$\delta^{18}$O$_{sugar}$ approach model 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 significantly \((r=0.79, p<0.001, n=20)\) with the actual \textit{modern-mean annual} RH values retrieved from GeoINTA (2012) \textit{for all investigated sites}, which generally validates the \(\delta^{18}O-\delta^2H\) conceptual model.

However, as depicted by Fig. 6, the reconstructed RH values systematically underestimate the actual \textit{modern-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 \(\delta^{18}O-\delta^2H\) 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, the reconstructed source water lies on the GMWL in the model, while local meteoric water lines and thus actual precipitation may have a d-excess offset from the GMWL \((d\text{-excess of GMWL} = 10 \, \text{‰})\). In our case, this effect should be negligible, as d-excess values of precipitation along the transect are only on the order of 4.8—11 \(\text{‰}\) (Bowen, 2012). Second, Third, given that leaf waxes \textit{are} considered to be formed mostly during early stages of leaf ontogeny (Kolattukudy, 1970; Riederer & Markstaedter, 1996; Kahmen et al., 2011a; Tipple et al., 2012) 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. As well, furthermore, \textit{compared to the mean annual (modern) RH values likely overestimate the RH values actually seen by leaves being photosynthetically active, growing season RH yields up to 9\% (CRU, 2013) lower values in Patagonia where seasonality is especially pronounced}. Indeed, when comparing the biomarker-based ‘reconstructed’ RH values with summer daytime RH values \textit{available for 6 stations along the investigated transect from www.ncds.noaa.gov}, the discrepancy becomes between ‘reconstructed’ and actual RH values becomes much smaller (Fig. 6). Fourth, reconstructed RH values will be also underestimated if \(n\)-alkanes do not fully incorporate the evaportranspirative \(\delta^2H\) enrichment...
of leaf water, which is the case for grasses (section 3.2; McInerney et al., 2011; Kahmen et al., 2013b). In the δ¹⁸O–δ²H diagram, leaf water would thus plot lower than the simple Craig–Gordon model predicts, and d-excess would be too negative.

Thirdly, the δ¹⁸O biosynthetic fractionation factor of ~+27 ‰, which has been reported for newly assimilated sugars and cellulose, may underestimate in our opinion the actual fractionation factor of hemicelluloses (Tuthorn et al., 2014; Zech M. et al., 2014a), which would result in reconstructed leaf water values plotting too far to the right in the δ¹⁸O–δ²H diagram (Fig. 5) and in turn to the observed underestimated RH values (Fig. 6). This can be explained We argue with the loss of a relatively ¹⁸O-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 is in agreement with the recent finding that about 80% of the oxygen atoms at the C-6 position are isotopically exchanged during cellulose synthesis point to a recent study of Waterhouse et al. (2013) who have determined the position specific δ¹⁸O values in cellulose. Further experimental studies as suggested and encouraged by Sternberg (2014) and Zech M. et al. (2014b) are urgently needed to ascertain an improved biosynthetic fractionation factor for hemicellulose-derived sugars. In contrast, the value of +27 ‰ would be an overestimation in cases where significant amounts of stem or root-derived sugars contribute to the soil sugar pool, because up to 40% of the oxygen atoms being biosynthesized in leaves are exchanging with non-enriched root/stem water during cellulose biosynthesis in roots/stems (Sternberg et al., 1986; Gessler et al., 2009). However, given that this such an exchange would result in an overestimation of reconstructed RH values (the opposite is observed, Fig. 6), we suggest that the majority of the sugar biomarkers along the topsoil transect investigated here are leaf-derived and not stem/root-derived.

3.3.2. Comparison of reconstructed and actual δ²Hₚₑₑₑ and δ¹⁸Oₚₑₑₑ values
Values of $\delta^{18}O_{\text{prec}}$ and $\delta^2H_{\text{prec}}$ reconstructed as the intercepts of the individual evaporation lines (EL) with the GMWL in the $\delta^{18}O$-$\delta^2H$ 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 $\delta^{18}O_{\text{prec}}$ and $\delta^2H_{\text{prec}}$, respectively) with the ‘actual’ $\delta^{18}O_{\text{prec}}$ and $\delta^2H_{\text{prec}}$ values as derived from Bowen (2012). While the reconstructed $\delta^{18}O_{\text{prec}}$ and $\delta^2H_{\text{prec}}$ values, like the reconstructed RH values, generally validate our conceptual model, they appear to systematically underestimate the actual $\delta^{18}O$ and $\delta^2H$ 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 $\delta^{18}O_{\text{prec}}$ and $\delta^2H_{\text{prec}}$ values. As well, the actual values for the isotopic composition of precipitation as $\delta^2H/\delta^{18}O_{\text{prec}}$ values used for our comparison with the biomarker-based ‘reconstructed values can be assumed to be one the uncertainty factor reported by While Bowen (2012) show-reported a confidence interval (95%) ranging from 0.2‰ to 1.2‰, and from 2‰ to 11‰ for $\delta^2H_{\text{prec}}$ and $\delta^{18}O_{\text{prec}}$, respectively. Future climate transect studies will be ideally carried out with actual precipitation being sampled for $\delta^2H/\delta^{18}O$ analyses. Moreover, we would like to emphasize also here the possible influence of seasonality. As reported for sugar biomarkers (Tuthorn et al., 2014), we suggest that leaf waxes as well likely reflect the humidity and the isotopic composition of spring and summer precipitation rather than mean annual values. Accounting for these seasonality effects, the apparent offsets would be reduced. Future modeling studies should therefore pay particular attention to seasonality, and consider using climate parameters of the growing season instead of annual means.

5. Conclusions

1 Please note that we chose here the term ‘actual’ for reasons of simplification in order to make the difference to the biomarker-based ‘reconstructed’ $\delta^{18}O_{\text{prec}}$ and $\delta^2H_{\text{prec}}$ values. Indeed, both the ‘reconstructed’ and the ‘actual’ values are derived from modeling, namely from our conceptual $\delta^2H_{\text{alkane}}$-$\delta^{18}O_{\text{sugar}}$ model and from Bowen’s (2012) online isotopes in precipitation calculator.
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 $\delta^2H$ values ranging from -155 to -222 ‰ and -128 to -225 ‰, respectively. These $\delta^2H$ values broadly parallel variations in the hydrogen isotopic composition of precipitation, but are modulated by evaporative $^2H$ enrichment of leaf water. A mechanistic leaf water model correctly simulates the overall trends. Sensitivity tests show that relative humidity exerts a stronger influence on evaporative enrichment than temperature.

Based on the premise that $n$-alkanes and hemicellulose biomarkers are primarily leaf-derived, we reconstruct $\delta^2H_{\text{leaf water}}$ and $\delta^{18}O_{\text{leaf water}}$, respectively, which in turn allows assessment of the d-excess of leaf water. The large calculated range in d-excess along the transect (-67 to -178 ‰) can be used to calculate modeled 'reconstructed' RH. ‘Reconstructed’ RH correlates significantly with empirical modern actual mean annual RH along the transect. Despite this overall correlation, we observe a systematic underestimation of 'reconstructed' RH, which we attribute to limitations of the combined $\delta^{18}O_{\text{sugar}}$/$\delta^2H_{\text{is}}$ model. Similarly, biomarker-based ‘reconstructed’ $\delta^{18}O_{\text{prec}}$ and $\delta^2H_{\text{prec}}$ values correlate high significantly with modern ‘actual’ precipitation.

The novel combined approach has great potential for paleohydrological and paleo-climate reconstructions. In principle, it allows determination of d-excess of past leaf water, thus constraining evaporative enrichment, as well as the isotopic signal of past precipitation.
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Fig. 3: Comparison of measured δ²Hn-alkanes (weighted mean of n-C29 and n-C31) and δ²Hfatty acids (weighted mean of n-C22, n-C24, n-C26, n-C28 and n-C30) pattern with δ²Hprec (Bowen, 2012) along the north-south climate transect (*min and *max representing annual minimum and maximum value at the sampling site). Additionally, assuming a consistent biosynthetic fractionation of -160 ‰ for the n-alkane and fatty acid biosynthesis in plants the biomarker-based ‘reconstructed’ isotopic composition of leaf water was inferred.

Fig. 4: Results of δ²Hleaf water model simulations and comparison with measured biomarker-based ‘reconstructed’ (assuming a biosynthetic fractionation factor of -160 ‰) isotopic composition of leaf water δ²Hn-alkane and δ²Hfatty based on n-alkanes and fatty acids, respectively. Sensitivity tests for δ²Hleaf water are shown for changes in RH and air temperature for all 20 sites along the transect.

Fig. 5: δ¹⁸O-δ²H diagram illustrating the conceptual model of the coupled δ²Hn-alkane-δ¹⁸O sugar approach (modified after Zech M. et al., 2013a), representing the global meteoric
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Fig. 7: Correlation of biomarker-based ‘reconstructed’ \( \delta^{18}O_{prec} \) and \( \delta^{2}H_{prec} \) values reconstructed from the biomarkers with actual modern ‘actual’ \( \delta^{18}O_{prec} \) and \( \delta^{2}H_{prec} \) values (from Bowen, 2012), a and b, respectively.