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Halle, 3rd of June 2015

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 δ^2H and δ^{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”)**

by M. Tuthorn, R. Zech, M. Ruppenthal, Y. Oelmann, A. Kahmen, H. F. del Valle, T. Eglinton, K. Rozanski and M. Zech.

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 δ^2H and δ^{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 _2Hlipid and _18Osugar 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 $\delta^2\mathrm{H}_{n\text{-alkane}}$ or $\delta^{18}\mathrm{O}_{\mathrm{sugar}}$ records, the proposed coupled $\delta^2\mathrm{H}_{n\text{-alkane}}$-$\delta^{18}\mathrm{O}_{\mathrm{sugar}}$ approach will allow a more robust reconstruction of $\delta^2\mathrm{H}/\delta^{18}\mathrm{O}_{\mathrm{precipitation}}$ 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 $\delta^{18}\mathrm{O}_{\mathrm{sugar}}$ 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 $\delta^2\mathrm{H}_{\mathrm{lipid}}$ 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 $\delta^2H_{n\text{-alkane}}$-$\delta^{18}O_{sugar}$ model is not based on the 20 samples analysed in this study for $\delta^2H_{n\text{-alkanes}}$ 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 $\delta^2H_{n\text{-alkane}}$-$\delta^{18}O_{sugar}$ 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 $\delta^2H_{n\text{-alkane}}$-$\delta^{18}O_{sugar}$ model is not based on the 20 samples analysed in this study for $\delta^2H_{n\text{-alkanes}}$ 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 $\delta^2H_{n\text{-alkane}}$-$\delta^{18}O_{sugar}$ 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 $\delta^{2}H_{n\text{-alkane}}$-$\delta^{18}O_{\text{sugar}}$ 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 ambitions 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 $\delta^{2}H_{n\text{-alkane}}$-$\delta^{18}O_{\text{sugar}}$ model (chapter 2.4. and 3.3.). Here, we reconstruct biomarker-based $\delta^{2}H/\delta^{18}O_{\text{leaf water}}$ values by correcting for biosynthetic fractionation factors. The reconstructed biomarker-based $\delta^{2}H/\delta^{18}O_{\text{leaf water}}$ 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 $\delta^2H_{n\text{-alkanes}}$ (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}) pattern 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 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 reprensented. 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: $\delta^{18}O$-δ^2H diagram illustrating the conceptual model of the coupled $\delta^2H_{n\text{-alkane}}$-$\delta^{18}O_{sugar}$ approach (modified after Zech M. et al., 2013a). $\delta^2H_{n\text{-alkane}}$ (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 $\delta^2H/\delta^{18}O_{\text{leaf water}}$ by subtracting the biosynthetic fractionation factors. The deuterium excess ($d = \delta^2H - 8 \times \delta^{18}O$) of leaf water serves as proxy for RH and $\delta^2H/\delta^{18}O_{prec}$ 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 δ^2H-$\delta^{18}O$ 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’ $\delta^2H/\delta^{18}O_{prec}$ with biomarker-based ‘reconstructed’ $\delta^2H/\delta^{18}O_{prec}$.

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.*
- *Huang, Y., Shuman, B., Wang, Y., and Webb, T.: Hydrogen isotope ratios of individual lipids in lake sediments as novel tracers of climatic and environmental change: a surface sediment test, J. Paleolimnol., 31, 363–375, 2004. : not cited in the text*
- *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 → a, b, or c ?*
- *P. 2472, line 3 : Tipple et al. → 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 → 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 sampleS…”?*
- *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

Mario Tuthorn & Michael Zech & Co-authors

Attachment: revised manuscript with track changes

Coupling~~ed~~ δ^2H and δ^{18}O~~isotopes of plant wax and hemicellulose markers~~ biomarker results~~cord~~ yields information on relative humidity and isotopic composition of precipitation – a climate transect validation study

M. Tuthorn[1], R. Zech[2], M. Ruppenthal[3], Y. Oelmann[3], A. Kahmen[4], H. F. del Valle[5], T. Eglinton[2], K. Rozanski[6], M. Zech[1,~~6~~7,*]

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*) Michael Zech, corresponding author (michael_zech@gmx.de)

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Abstract

The δ^{2}H isotopic composition of leaf waxes, especially of *n*-alkanes ($\delta^{2}H_{n\text{-alkanes}}$) 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 $\delta^{2}H_{n\text{-alkane}}$-$\delta^{18}O_{sugar}$ biomarker approach. This coupling allows~~We analyzed δ^{2}H on *n*-alkanes and fatty acids in topsoils along a climate transect in Argentina, for which we had previously measured δ^{18}O on plant-derived sugars. Our results indicate that leaf wax biomarker δ^{2}H values ($\delta^{2}H_{lipids}$) primarily reflect $\delta^{2}H_{source\ water}$ (precipitation), but are modulated by evapotranspirative enrichment. A mechanistic model is able to produce the main trends in $\delta^{2}H_{lipids}$ 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 $\delta^{2}H_{lipid}$ and $\delta^{18}O_{sugar}$ biomarker results and (ii) application of biosynthetic fractionation factors allows~~ ~~calculating~~ calculating biomarker-based/-reconstructed~~the~~ (i) δ^{2}H~~-~~/$\delta^{18}O_{leaf\ water}$ values, ~~isotopic composition of leaf water along the transect. This also yields~~(ii) ~~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) δ^{2}H/$\delta^{18}O_{precipitation}$~~.~~ values. Here were present a respective climate transect validation study by coupling new results from δ^{2}H analyses on *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 ~~Correlating the~~ reconstructed RH and δ^{2}H/$\delta^{18}O_{precipitation}$ values correlate highly significantly with actual RH and δ^{2}H/$\delta^{18}O_{precipitation}$ values. We conclude that compared to single $\delta^{2}H_{n\text{-alkane}}$ or $\delta^{18}O_{sugar}$ records, the proposed coupled $\delta^{2}H_{n\text{-alkane}}$-$\delta^{18}O_{sugar}$ approach will allow more robust ~~to the measured RH, as well as the reconstructed and actual isotopic composition of~~

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~~precipitation, we tested the value of combined δ^2H and δ^{18}O approach for the reconstruction of~~ δ^2H/δ^{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 δ^2H and δ^{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 δ^2H and δ^{18}O of precipitation along the transect lends support to the coupled δ^2H$_{lipid}$ and δ^{18}O$_{sugar}$ biomarker approach for future paleoclimate research.~~

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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 M. et al., 2011b). The δ^2H 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 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, ~~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 ^{2}H 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 ^{2}H 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 δ^2H biomarker records alone.~~

~~Compound-specific δ^2H of lipid biomarkers, especially *n*-alkanes due to their good preservation, are already widely applied in paleoclimate and -hydrological research (Sauer et al., 2001; Schefuss et al., 2005; Pagani et al., 2006; Tierney et al., 2008; Zech et al., 2013c~~)~~.~~ a robust reconstruction of δ^2H_{prec} from soils and sedimentary records turns increasingly out to be quite challenging, because it is hitherto difficult to disentangle past changes in δ^2H_{prec} and

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changes in evapotranspirative enrichment of leaf water (Zech, R. et al., 2013; Zech, M. et al., 2015). ~~Comparison of $\delta^2H_{n\text{-alkanes}}$ with $\delta^2H_{\text{fatty acids}}$ 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 δ^2H analyses, compound-specific $\delta^{18}O$ analyses ~~of sugars remain in their infancy, yet hold significant promise~~are by far less adopted by the scientific community, so far (Hener 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 ($\delta^{18}O_{\text{sugars}}$) 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 $\delta^2H_{n\text{-alkane}}$-$\delta^{18}O_{\text{sugar}}$ model for paleoclimate research and suggested that this coupling allows overcoming the above defined limitation of single $\delta^2H_{n\text{-alkane}}$ approaches. Accordingly, the coupled $\delta^2H_{n\text{-alkane}}$-$\delta^{18}O_{\text{sugar}}$ approach allows reconstructing (i) $\delta^2H/\delta^{18}O_{\text{leaf water}}$ 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) $\delta^2H/\delta^{18}O_{\text{prec}}$ values. ~~Given the evaporative ^2H and ^{18}O enrichment of leaf water being recorded in both, *n*-alkanes and hemicellulose-derived sugars the combined plant wax δ^2H 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 δ^2H-$\delta^{18}O$ diagram,~~

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~~sugar and *n*-alkane isotopic information may enable reconstruction of the isotopic composition of precipitation in paleohydrological studies (Zech et al., 2013a).~~

The~~is~~ here presented study ~~describes the application of a combined~~aimed at validating or falsifying the coupled δ^{2}H and δ^{18}O biomarker approach by applying it to a modern topsoil~~s sampled along a~~ climate transect from~~in~~ Argentina. ~~The aims of this investigation are to~~More specifically, we aimed at (i)

~~(i)~~ analysing and compar~~e~~ing the δ^{2}H values of *n*-alkanes ~~with those of~~and fatty acids, (ii) modelling~~ed~~ ~~*n*-alkane~~ leaf water enrichment along the transect and comparison with δ^{2}H$_{n\text{-alkanes}}$ and δ^{2}H$_{\text{fatty acids}}$ ~~values and δ^{18}O values of sugars and evaluate the dominant climate factors influencing these values~~,

(iii) reconstructing d-excess of leaf water using the coupled δ^{2}H$_{n\text{-alkane}}$~~- values of *n*-alkanes and~~ δ^{18}O~~ values of~~$_{\text{sugar}}$~~s~~ approach and ~~assess~~ evaluating the potential ~~of~~ for reconstructing~~ed~~ ~~d-excess of leaf water as paleoclimate proxy for~~ RH, and

(iv~~ii~~) reconstructing ‘biomarker-based’ δ^{2}H/δ^{18}O$_{\text{prec}}$ values and ~~evaluate the potential of the combined δ^{18}O and δ^{2}H biomarker approach to reconstruct th~~comparison with actual δ^{2}H/δ^{18}O$_{\text{prec}}$ values.~~e 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~~ locations 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 δ^{18}O and δ^{2}H values (δ^{18}O$_{\text{prec}}$ and δ^{2}H$_{\text{prec}}$, respectively) (Bowen, 2012~~; GeoINTA, 2012~~).

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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 $\delta^{18}O_{sugar}$ 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.

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 dichloromethane (DCM) and methanol (MeOH; 9:1) according to Zech R. et al. (2013~~e~~). 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

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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~~d~~ 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). 5α 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 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 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 $\delta^2H_{\text{leaf water}}$ 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 (Δ^2H_e) at the evaporative surface in the mesophyll is given by the equation:

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

where ε^+ is the equilibrium fractionation between liquid water and vapor at the air-water interfaces~~ (Bottinga and Craig, 1969)~~, 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 (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):

$$\Delta^2 H_{leafwater} = \frac{\Delta^2 H_e\left(1 - e^{-\wp}\right)}{EL/CD}. \qquad \text{(Eqn. 2)}$$

The quotient of EL/CD represents the Péclet number ($\wp$) 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 ^{1}~~^{2}~~H^{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 δ^2H. This simplified model is based on the assumption that leaf temperature equals air temperature and that atmospheric vapor δ^2H is in

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Formatiert: Nicht Hochgestellt/ Tiefgestellt 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. 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 δ^2H values we obtained the model input variables air temperature, atmospheric vapor pressure and source water δ^2H from GeoINTA (2012) and Bowen (2012), respectively. The isotopic composition of the leaf water can be estimated according to Eqn. 3:

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

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. ~~The c~~Conceptual model for a coupled $\delta^{18}O$-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 word-wide typically plots along/close to the so-called global meteoric water line (GMWL, $\delta^2H = 8 \times \delta^{18}O + 10$) in a $\delta^{18}O$-δ^2H diagram (Dansgaard, 1964) (Fig. 5). Due to fractionation processes, evaporation/transpiration causes ~~evaporating~~ water vapour to be isotopically depleted in ^{18}O and ^2H, whereas residual (leaf) water (δ^2H/$\delta^{18}O_{\text{leaf water}}$) is isotopically enriched. ~~–~~In a $\delta^{18}O$-δ^2H diagram, leaf water therefore does not plot on the GMWL but on an evaporation line (EL). ~~t~~The distance of ~~reconstructed~~ leaf water to the Global Meteoric Water Line (GMWL) can be de~~fines the~~scribed as deuterium excess (d = $\delta^2H - 8\cdot\delta^{18}O$)~~. Observed deviation is caused by the kinetic effect resulting in slower diffusivity of the $^1H^1H^{18}O$ molecules compared to the $^2H^1H^{16}O$ molecules. .More humid conditions and less evapotranspiration are reflected by~~

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~~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., 2013~~c~~):

$$RH = 1 - \frac{\Delta d}{\left(\varepsilon_2^* - 8\cdot\varepsilon_{18}^* + C_k^2 - 8\cdot C_k^{18}\right)} \qquad \text{(Eqn. 4)}$$

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 $\delta^2H_{n\text{-alkane}}$-$\delta^{18}O_{sugar}$ approach allows reconstructing RH values.

~~The combined $\delta^{18}O$-δ^2H biomarker~~ The biomarker-based reconstructed $\delta^2H/\delta^{18}O_{leaf\ water}$ values ~~approach also~~ allow~~s~~ furthermore reconstructi~~on~~ng ~~of~~ the isotopic composition of plant source water, which can be considered ~~as~~ in an approximation to reflect~~for~~ δ^2H/~~prec and~~ $\delta^{18}O_{prec}$~~.~~ (illustrated ~~In Figure 5 these are given by the~~as intercepts of the individual ~~evaporation lines (~~ELs~~)~~ with the GMWL in Fig. 5). 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 $\delta^{18}O$-δ^2H biomarker results the reader is referred to Zech M. et al. (2013~~a~~).

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3. Results and Discussion

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

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 much more negative δ^2H values in the south 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 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 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. Especially in the middle part of the transect, δ^2H 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 δ^2H of long chain *n*-alkanes in further discussion and calculations.

3.2. Evapotranspirative ^{2}H 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 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-chained fatty acids synthesized mostly by unicellular/multicellular marine alge. By contrast, there are hardly any biosynthetic fractionation factors reported for long-chained 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 ^{2}H 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 $\delta^2H_{\text{leaf water}}$ values inferred from the measured *n*-alkanes and fatty acids, and $\delta^2H_{\text{leaf water}}$ values calculated using the Pecletmodified 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 δ^{2}H along the transect with reasonable accuracy, the model does not capture site-to-site excursions in the *n*-alkane-derived leaf water δ^{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~~could explain the underestimation of the modeled $\delta^{2}\text{H}_{\text{leaf water}}$ values in the middle part of the transect (Fig. 4). In contrast, the model might overestimate $\delta^{2}\text{H}_{\text{leaf water}}$ 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 δ^{2}H isotopic composition of leaf water, changes in RH yield difference of up to ~30 ‰ (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 δ^{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 $\delta^{2}H_{n\text{-alkane}}$ and $\delta^{18}O_{sugar}$ ~~and $\delta^{2}H$ *n*-alkane~~ biomarker ~~analyses~~results

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~~A~~ The conceptual model for the ~~combined interpretation of~~coupled $\delta^{2}H_{n\text{-alkane}}$ ~~and~~ - $\delta^{18}O_{sugar}$ biomarker approach~~s~~ ~~can be~~is illustrated in ~~a $\delta^{18}O$-$\delta^{2}H$ 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’ $\delta^{2}H/\delta^{18}O_{leaf\ water}$ values can be calculated from the ~~isotopic composition of the~~ biomarkers by applying biosynthetic fractionation factors ε_{bio}. ~~an average value according to various studies of the biosynthetic fractionation factors resulting in~~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 $\delta^{2}H$ and $\delta^{18}O$, respectively (Fig. 5).

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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 $\delta^{2}H_{n\text{-alkane}}$ - $\delta^{18}O_{sugar}$ approach ~~model~~ range from 16 to 65 %, with one extremely low value of 5 % (Fig. 6). Reconstructed RH values follow the

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systematic d-excess trend and correlate significantly (r=0.79, p<0.001, n=20) with the actual ~~modern~~ mean annual RH values retrieved from GeoINTA (2012) for all investigated sites.~~,~~ ~~which generally validates the δ^{18}O-δ^{2}H conceptual model.~~

However, as depicted by Fig. 6, the reconstructed RH values systematically underestimate the actual ~~(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 δ^{18}O-δ^{2}H 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-excess of GMWL = 10 ‰). 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 ‰ (Bowen, 2012).~~ Second~~Third~~, given that leaf waxes are considered to be formed mostly during early stages of leaf ontogeny (Kolattukudy, 1970; Riederer & Markstaedter, 1996; Kahmen et al., 2011a; Tipple et al., 2013~~2~~) 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, ~~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 (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 evapotranspirative ^{2}H enrichment~~

~~of leaf water, which is the case for grasses (section 3.2; McInerney et al., 2011; Kahmen et al., 2013b). In the δ^{18}O-δ^{2}H diagram, leaf water would thus plot lower than the simple Craig-Gordon model predicts, and d-excess would be too negative.~~

Third~~Finally~~, the δ^{18}O biosynthetic fractionation factor of ~+27 ‰, which has been reported for newly assimilated sugars and cellulose, ~~may~~ underestimates in our opinion the actual fractionation factor of hemicelluloses (Tuthorn et al., 2014; Zech M. et al., 2014a).~~,~~ ~~which~~ This ~~would~~ results in reconstructed leaf water values plotting too far to the right in the δ^{18}O-δ^{2}H 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 ^{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 ~~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 δ^{18}O 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 thissuch 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 δ^{2}H$_{prec}$ and δ^{18}O$_{prec}$ values

Values of $\delta^{18}O_{prec}$ and $\delta^{2}H_{prec}$ reconstructed as the intercepts of the individual evaporation lines (EL) with the GMWL in the $\delta^{18}O$-$\delta^{2}H$ 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 $\delta^{2}H_{prec}$, respectively) with the 'actual'[1] $\delta^{2}H_{prec}$ and $\delta^{18}O_{prec}$ values as derived from Bowen (2012). While the reconstructed $\delta^{18}O_{prec}$ and $\delta^{2}H_{prec}$ values, like the reconstructed RH values, generally validate our conceptual model, they appear to systematically underestimate the actual $\delta^{18}O$ and $\delta^{2}H$ 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 $\delta^{2}H_{prec}$ values. ~~As well, the actual values for the isotopic composition of precipitation as~~Hence, the 'actual' $\delta^{2}H/\delta^{18}O_{prec}$ 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 $\delta^{2}H_{prec}$ and $\delta^{18}O_{prec}$, respectively, future climate transect studies will be ideally carried out with actual precipitation being sampled for $\delta^{2}H/\delta^{18}O$ analyses. Moreover~~Here~~, we would like to emphasize also here the ~~possible~~very likely influence of seasonality. As reported for sugar biomarkers (Tuthorn et al., 2014), we suggest that also leaf waxes ~~as well likely~~ mainly 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

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[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' $\delta^{18}O_{prec}$ and $\delta^{2}H_{prec}$ values. Indeed, both the 'reconstructed' and the 'actual' values are derived from modelling, namely from our conceptual $\delta^{2}H_{n\text{-alkane}}$-$\delta^{18}O_{sugar}$ model and from Bowen's (2012) online isotopes in precipitation calculator.

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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 $\delta^{2}\text{H}$ values ranging from -155 to -222 ‰ and -128 to -225 ‰, respectively. These $\delta^{2}\text{H}$ 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 a stronger influence on evaporative enrichment than temperature.

Based on the premise that *n*-alkanes and hemicellulose biomarkers are primarily leaf-derived, we reconstruct $\delta^{2}\text{H}_{\text{leaf water}}$ and $\delta^{18}\text{O}_{\text{leaf water}}$, 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~~/model~~ biomarker-based ‘reconstructed’ RH.~~;~~ ~~modeled~~ ‘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 $\delta^{18}\text{O}$-$\delta^{2}\text{H}$~~ is, however, largely reduced when comparing with actual summer daytime RH values~~conceptual model~~. Similarly, biomarker-based ‘reconstructed’ $\delta^{18}\text{O}_{\text{prec}}$ and $\delta^{2}\text{H}_{\text{prec}}$ ~~calculated/modeled from biomarker isotopic compositions significantly~~values correlate highly significantly with ~~modern~~ ‘actual’ precipitation.

~~The novel combined~~We conclude that compared to single $\delta^{2}\text{H}_{n\text{-alkane}}$ or $\delta^{18}\text{O}_{\text{sugar}}$ records, the proposed coupled $\delta^{2}\text{H}_{n\text{-alkane}}$-$\delta^{18}\text{O}_{\text{sugar}}$ approach will allow more robust $\delta^{2}\text{H}/\delta^{18}\text{O}_{\text{prec}}$ reconstructions and additionally the reconstruction of summer daytime RH changes/history using d-excess of leaf water as proxy in future paleoclimate studies. ~~$\delta^{2}\text{H}$-$\delta^{18}\text{O}$ biomarker approach has great potential for paleo-hydrological 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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References

Allison, G.B., Gat, J.R., Leaney, F.W.J., 1985. The relationship between deuterium and oxygen-18 delta values in leaf water. Chemical Geology 58, 145-156.

Altermatt, H.A., Neish, A.C., 1956. The biosynthesis of cell wall carbohydrates: III. further studies on formation of cellulose and xylan from labeled monosaccharides in wheat plants. Canadian Journal of Biochemistry and Physiology 34, 405-413.

Araguas-Araguas, L., Froehlich, K., Rozanski, K., 2000. Deuterium and oxygen-18 isotope composition of precipitation and atmospheric moisture. Hydrological Processes 14, 1341-1355.

Bariac, T., Gonzales-Dunia, J., Katerji, N., Bethenod, O., Bertolini, J.M., Mariotti, A., 1994. Spatial variation of the isotopic composition of water (^{18}O, ^{2}H) in the soil-plant-atmosphere system. Chemical Geology 115, 317-333.

Bianchi, T., Canuel, E.A., 2011. Chemical Biomarkers in Aquatic Ecosystems. Princeton University Press, Princeton.

~~Bottinga, Y., Craig, H., 1969. Oxygen isotope fractionation between CO2 and water, and the isotopic composition of marine atmospheric CO2. Earth and Planetary Science Letters 5, 285-295.~~

Bowen, G.J., 2012 The Online Isotopes in Precipitation Calculator, version 2.2. http://www.waterisotopes.org.

Bull, I.D., Nott, C.J., van Bergen, P.F., Poulton, P.R., Evershed, R.P., 2000. Organic geochemical studies of soils from the Rothamsted classical experiments - VI. The occurrence and source of organic acids in an experimental grassland soil. Soil Biology and Biochemistry 32, 1367-1376.

Burget, E., Verma, R., Mølhøj, M., Reiter, W., 2003. The biosynthesis of L-arabinose in plants: molecular cloning and characterization of a golgi-localized UDP-D-xylose 4-epimerase encoded by the MUR4 gene of arabidopsis. The Plant Cell 15, 523-531.

Burgos, J.J., Vidal, A.L., 1951. Los climas de la República Argentina, segun la nueva clasificación de Thornthwaite. Meteoros 1, 1-32.

Cernusak, L.A., Wong, S.C., Farquhar, G.D., 2003. Oxygen isotope composition of phloem sap in relation to leaf water in *Ricinus communis*. Functional Plant Biology 30, 1059-1070.

Chikaraishi, Y., Naraoka H., 2007. δ^{13}C and δD relationships among three n-alkyl compound classes (n-alkanoic acid, n-alkane and n-alkanol) of terrestrial higher plants. Organic Geochemistry 38, 198-215.

Craig, H., Gordon, L.I., 1965 Deuterium and oxygene-18 variations in the ocean and the marine atmosphere. In: Conference on Stable Isotopes in Oceanographic Studies and Paleotemperatures (Ed. by E. Tongiorgi), pp. 9-130, Spoleto, Italy.

~~CRU, 2013 University of East Anglia Climate Research Unit, Relative humidity data. Available at: http://www.cru.uea.ac.uk/cru/data/hrg/tmc/. Accessed 28.11.2013, 2013.~~

Dongmann, G., Nürnberg, H.W., Förstel, H., Wagener, K., 1974. On the enrichment of $H_2^{18}O$ in the leaves of transpiring plants. Radiation and Environmental Biophysics 11, 41-52.

Douglas, P.M.J., Pagani, M., Brenner, M., Hodell, D.A., Curtis, J.H., 2012. Aridity and vegetation composition are important determinants of leaf-wax δD values in southeastern Mexico and Central America. Geochimica et Cosmochimica Acta 97, 24-45.

Eglinton, G., Hamilton, R.J., 1967. Leaf Epicuticular Waxes. Science 156, 1322-1335.

Eglinton, T.I., Eglinton, G., 2008. Molecular proxies for paleoclimatology. Earth and Planetary Science Letters 275, 1-16.

Ehleringer, J.R., Dawson, T.E., 1992. Water uptake by plants: perspectives from stable isotope composition. Plant, Cell & Environment 15, 1073-1082.

Farquhar, G.D., Cernusak, L.A., Barnes, B., 2007. Heavy Water Fractionation during Transpiration. Plant Physiology 143, 11-18.

Farquhar, G.D., Lloyd, J., 1993 Carbon and oxygene isotope effects in the exchange of carbon dioxide between terrestrial plants and the atmosphere, In: J.R. Ehleringer, A.E. Hall, G.D. Farquhar (Eds.), Stable isotopes and plant carbon-water relations. Academic Press, Inc., San Diego, pp. 47-70.

Feakins, S.J., Sessions, A.L., 2010. Controls on the D/H ratios of plant leaf waxes in an arid ecosystem. Geochimica et Cosmochimica Acta 74, 2128-2141.

Fernández, O.A., Busso, C.A., 1997 Arid and semi-arid rangelands: two thirds of Argentina, pp. 41-60. RALA Report 200.

Flanagan, L.B., Comstock, J.P., Ehleringer, J.R., 1991. Comparison of modeled and observed environmental influences on the stable oxygen and hydrogen isotope composition of leaf water in *Phaseolus vulgaris* L. Plant Physiology 96, 588-596.

Gamarra, B., Kahmen, A., 2015. Concentrations and d2H values of cuticular n-alkanes vary significantly among plant organs, species and habitats in grasses from alpine and a temperated European grassland. Oecologia DOI 10.1007/s00442-015-3278-6.

Gat, J.R., 1996. Oxygen and Hydrogen Isotopes in the Hydrologic Cycle. Annual Review of Earth and Planetary Sciences 24, 225-262.

Gat, J.R., Bowser, C., 1991 The heavy isotope enrichment of water in coupled evaporative systems, In: H.P. Taylor, J.R. O'Neil, I.R. Kaplan (Eds.), Stable Isotope Geochemistry: A Tribute to Samuel Epstein. The Geochemical Society, Lancester, pp. 159-168.

GeoINTA, 2012 Instituto Nacional de Technologia Agropecuaria Visualizador Integrado. Available online at: http://geointa.inta.gov.ar/visor/ . Accessed 01.08.2012.

Gessler, A., Brandes, E., Buchmann, N., Helle, G., Rennenberg, H., Barnard, R.L., 2009. Tracing carbon and oxygen isotope signals from newly assimilated sugars in the leaves to the tree-ring archive. Plant, Cell & Environment 32, 780-795.

Greule, M., Hänsel, C., Bauermann, U., Mosandl, A., 2008. Feed additives: authenticity assessment using multicomponent-/multielement-isotope ratio mass spectrometry. European Food Research and Technology 227, 767-776.

Häggi, C., Zech, R., McIntyre, C., Zech, M. and Eglinton, T., 2014. On the stratigraphic integrity of leaf-wax biomarkers in loess paleosol. Biogeosciences 11, 2455-2463.

Harper, A., Bar-Peled, M., 2002. Biosynthesis of UDP-xylose. Cloning and characterization of a novel Arabidopsis gene family, UXS, encoding soluble and putative membrane-bound UDP-glucuronic acid decarboxylase isoforms. Plant Physiology 130, 2188-2198.

Hener, U., Brand, W.A., Hilkert, A.W., Juchelka, D., Mosandl, A., Podebrad, F., 1998. Simultaneous on-line analysis of 18O/16O and 13C/12C ratios of organic compounds using GC-pyrolysis-IRMS. Zeitschrift für Lebensmitteluntersuchung und -Forschung A 206, 230-232.

Formatiert: Englisch (Großbritannien)

Formatiert: Englisch (Großbritannien)

Formatiert: Deutsch (Deutschland)

Formatiert: Block

Horita, J., Wesolowski, D.J., 1994. Liquid-vapor fractionation of oxygen and hydrogen isotopes of water from the freezing to the critical temperature. Geochimica et Cosmochimica Acta 58, 3425-3437.

Huang, Y., Shuman, B., Wang, Y., Webb, T., 2004. Hydrogen isotope ratios of individual lipids in lake sediments as novel tracers of climatic and environmental change: a surface sediment test. Journal of Paleolimnology 31, 363-375.

Juchelka, D., Beck, T., Hener, U., Dettmar, F., Mosandl, A., 1998. Multidimensional Gas Chromatography Coupled On-Line with Isotope Ratio Mass Spectrometry (MDGC-IRMS): Progress in the Analytical Authentication of Genuine Flavor Components. Journal of High Resolution Chromatography 21, 145-151.

Jung, J., Puff, B., Eberts, T., Hener, U., Mosandl, A., 2007. Reductive ester cleavage of acyl glycerides-GC-C/P-IRMS measurements of glycerol and fatty alcohols. European Food Research and Technology 225, 191-197.

Jung, J., Sewenig, S., Hener, U., Mosandl, A., 2005. Comprehensive authenticity assessment of lavender oils using multielement/multicomponent isotope ratio mass spectrometry analysis and enantioselective multidimensional gas chromatography-mass spectrometry. European Food Research and Technology 220, 232-237.

Kahmen, A., Dawson, T.E., Vieth, A., Sachse, D., 2011a. Leaf wax *n*-alkane δD values are determined early in the ontogeny of *Populus trichocarpa* leaves when grown under controlled environmental conditions. Plant, Cell & Environment 34, 1639-1651.

Kahmen A., Sachse D., Arndt S.K., Tu K.P., Farrington H., Vitousek P.M., and Dawson T.E., 2011b. Cellulose $\delta^{18}O$ is an index of leaf-to-air vapor pressure difference (VPD) in tropical plants. Proceedings of the National Academy of Sciences 108, 1981-1986.

Kahmen, A., Hoffmann, B., Schefuss, E., Arndt, S.K., Cernusak, L.A., West, J.B., Sachse, D., 2013a. Leaf water deuterium enrichment shapes leaf wax *n*-alkane δD values of angiosperm plants II: Observational evidence and global implications. Geochimica et Cosmochimica Acta 111, 50-63.

Kahmen, A., Schefuß, E., Sachse, D., 2013b. Leaf water deuterium enrichment shapes leaf wax *n*-alkane δD values of angiosperm plants I: Experimental evidence and mechanistic insights. Geochimica et Cosmochimica Acta 111, 39-49.

Kahmen, A., Simonin K., Tu, K., Goldsmith, G.R., Dawson, T.E., 2009. The influence of species and growing conditions on the 18-O enrichment of leaf water and its impact on 'effective path length'. New Phytologist, 184, 619-630.

Kahmen, A., Simonin, K., Tu, K.P., Merchant, A., Callister, A., Siegwolf, R., Dawson, T.E., Arndt, S.K., 2008. Effects of environmental parameters, leaf physiological properties and leaf water relations on leaf water $\delta^{18}O$ enrichment in different Eucalyptus species. Plant, Cell & Environment 31, 738-751.

Kolattukudy, P., 1970. Cutin biosynthesis in *Vicia faba* leaves - effect of age. Plant Physiology 46, 759-760.

Le Houérou, H.N., 1996. Climate change, drought and desertification. Journal of Arid Environments 34, 133-185.

Matsumoto, K., Kawamura, K., Uchida, M., Shibata, Y., 2007. Radiocarbon content and stable carbon isotopic ratios of individual fatty acids in subsurface soils: Implication for selective microbial degradation and modification of soil organic matter. Geochemical Journal 41, 483-492.

McInerney, F.A., Helliker, B.R., Freeman, K.H., 2011. Hydrogen isotope ratios of leaf wax *n*-alkanes in grasses are insensitive to transpiration. Geochimica et Cosmochimica Acta 75, 541-554.

Merlivat, L., 1978. Molecular diffusivities of $H_2^{16}O$,$HD^{16}O$, and $H_2^{18}O$ in gases. The Journal of Chemical Physics 69, 2864-2871.

Nguyen Tu, T.T., Egasse, C.l., Zeller, B., Bardoux, G.r., Biron, P., Ponge, J.-F.o., David, B., Derenne, S., 2011. Early degradation of plant alkanes in soils: A litterbag experiment using 13C-labelled leaves. Soil Biology and Biochemistry 43, 2222-2228.

Pagani, M., Pedentchouk, N., Huber, M., Sluijs, A., Schouten, S., 2006. Arctic hydrology during global warming at the Paleocene/Eocene thermal maximum. Nature 442, 671-675.

Paruelo, J.M., Beltrán, A., Jobbágy, E., Sala, O.E., Golluscio, R.A., 1998. The climate of Patagonia: general patterns and controls on biotic processes. Ecologia Austral 8, 85-101.

Formatiert: Englisch (Großbritannien)

Formatiert: Schriftart: Kursiv

Formatiert: Deutsch (Deutschland)

Formatiert: Schriftart: Kursiv

Formatiert: Schriftart: Kursiv

Formatiert: Schriftart: Kursiv

Paul, D., Skrzypek, G., Fórizs, I., 2007. Normalization of measured stable isotopic compositions to isotope reference scales - a review. Rapid Communications in Mass Spectrometry 21, 3006-3014.

Pedentchouk, N., Sumner, W., Tipple, B., Pagani, M., 2008. delta C-13 and delta D compositions of *n*-alkanes from modern angiosperms and conifers: An experimental set up in central Washington State, USA. Organic Geochemistry 39, 1066-1071.

Radke, J., Bechtel, A., Gaupp, R., Püttmann, W., Schwark, L., Sachse, D., Gleixner, G., 2005. Correlation between hydrogen isotope ratios of lipid biomarkers and sediment maturity. Geochimica et Cosmochimica Acta 69, 5517-5530.

Rao, Z., Zhu, Z., Jia, G., Henderson, A.C.G., Xue, Q., Wang, S., 2009. Compound specific δD values of long chain *n*-alkanes derived from terrestrial higher plants are indicative of the δD of meteoric waters: Evidence from surface soils in eastern China. Organic Geochemistry 40, 922-930.

Riederer, M., Markstaedter, C., 1996 Cuticular waxes: a critical assessment of current knowledge, In: K.G. Kerstiens (Ed.), Plant Cuticules - An Integrated Functional Approach. BIOS Scientific Publishers, Oxford.

Rozanski, K., Araguas-Araguas, L., Gonfiantini, R., 1993. Isotopic patterns in modern global precipitation. In: P.K. Swart et al. (Editor), Climate Change in Continental Isotopic Records. Geophysical Monograph 78, American Geophysical Union, Washington, DC 20009, USA, pp. 1-37.

Ruppenthal, M., Oelmann, Y., Francisco del Valle, H., Wilcke, W., 2015. Stable isotope ratios of nonexchangeable hydrogen in organic matter of soils and plants along a 2100-km climosequence in Argentina: New insights into soil organic matter sources and transformations? Geochimica Et Cosmochimica Acta 152:54-71

Sachse, D., Billault, I., Bowen, G.J., Chikaraishi, Y., Dawson, T.E., Feakins, S.J., Freeman, K.H., Magill, C.R., McInerney, F.A., van der Meer, M.T.J., Polissar, P., Robins, R.J., Sachs, J.P., Schmidt, H.-L., Sessions, A.L., White, J.W.C., West, J.B., Kahmen, A., 2012. Molecular Paleohydrology: Interpreting the Hydrogen-Isotopic Composition of Lipid Biomarkers from Photosynthesizing Organisms. Annual Review of Earth and Planetary Sciences 40, 221-249.

Sachse, D., Radke, J., Gleixner, G., 2004. Hydrogen isotope ratios of recent lacustrine sedimentary *n*-alkanes record modern climate variability. Geochimica et Cosmochimica Acta 68, 4877-4889.

Sachse, D., Radke, J., Gleixner, G., 2006. δD values of individual *n*-alkanes from terrestrial plants along a climatic gradient - Implications for the sedimentary biomarker record. Organic Geochemistry 37, 469-483.

Samuels, L., Kunst, L., Jetter, R., 2008. Sealing Plant Surfaces: Cuticular Wax Formation by Epidermal Cells. Annual Review of Plant Biology 59, 683-707.

Sauer, P.E., Eglinton, T.I., Hayes, J.M., Schimmelmann, A., Sessions, A.L., 2001. Compound-specific D/H ratios of lipid biomarkers from sediments as a proxy for environmental and climatic conditions. Geochimica et Cosmochimica Acta 65, 213-222.

Schefuss, E., Schouten, S., Schneider, R.R., 2005. Climatic controls on central African hydrology during the past 20,000 years. 437, 1003-1006.

Schmidt, H.-L., Werner, R.A., Rossmann, A., 2001. ^{18}O pattern and biosynthesis of natural plant products. Phytochemistry 58, 9-32.

Sessions, A.L., Burgoyne, T.W., Schimmelmann, A., Hayes, J.M., 1999. Fractionation of hydrogen isotopes in lipid biosynthesis. Organic Geochemistry 30, 1193-1200.

Smith, F.A., Freeman, K.H., 2006. Influence of physiology and climate on δD of leaf wax *n*-alkanes from C3 and C4 grasses. Geochimica et Cosmochimica Acta 70, 1172-1187.

Song X., Barbour M.M., Farquhar G.D., Vann D.R., Helliker B.R.,2013. Transpiration rate relates to within- and across-species variations in effective path length in a leaf water model of oxygene isotope enrichment. Plant, Cell and Environment 36, 1338-1351.

Sternberg, L., DeNiro, M.J., Savidge, R.A., 1986. Oxygen isotope exchange between metabolites and water during biochemical reactions leading to cellulose synthesis. Plant Physiology 82, 423-427.

Sternberg, L., 2014. Comment on “Oxygen isotope ratios (^{18}O/^{16}O) of hemicellulose-derived sugar biomarkers in plants, soils and sediments as paleoclimate proxy I: Insight from a climate chamber experiment” by Zech et al. (2014). Geochim Cosmochim Acta 141, 677-679.

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Formatiert: Schriftart: 11 Pt., Englisch (Großbritannien)

Formatiert: Schriftart: 11 Pt.

Tierney, J.E., Russel, J.M., Huang, Y., Sinninghe Damsté, J.S., Hopmans, E.C., Cohen, A.S., 2008. Northern Hemisphere Controls on Tropical Southeast African Climate During the Past 60,000 Years. Science 322, 252-255.

Tipple, B.J., Berke, M.A., Doman, C.E., Khachaturyan, S., Ehleringer, J.R., 2013. Leaf-wax *n*-alkanes record the plant-water environment at leaf flush. Proceedings of National Academy of Science 110, 2659-2664.

Tuthorn, M., Zech, M., Ruppenthal, M., Oelmann, Y., Kahmen, A., Valle, H.c.F.d., Wilcke, W., Glaser, B., 2014. Oxygen isotope ratios (18O/16O) of hemicellulose-derived sugar biomarkers in plants, soils and sediments as paleoclimate proxy II: Insight from a climate transect study. Geochimica et Cosmochimica Acta 126, 624-634.

Walker, C.D., Brunel, J.-P., 1990. Examining evapotranspiration in a semi-arid region using stable isotopes of hydrogen and oxygen. Journal of Hydrology 118, 55-75.

Waterhouse, J.S., Cheng, S., Juchelka, D., Loader, N.J., McCarroll, D., Switsur, V.R., Gautam, L., 2013. Position-specific measurement of oxygen isotope ratios in cellulose: Isotopic exchange during heterotrophic cellulose synthesis. Geochimica et Cosmochimica Acta 112, 178-191.

~~Werner, R., 2003. The online ^{18}O/^{16}O analysis: development and application. Isotopes in Environmental and Health Studies 39, 85-104.~~

Yakir, D., 1992. Variations in the natural abundance of oxygen-18 and deuterium in plant carbohydrates. Plant, Cell & Environment 15, 1005-1020.

Yakir, D., DeNiro, M.J., 1990. Oxygen and hydrogen isotope fractionation during cellulose metabolism in *Lemna gibba* L. Plant Physiology 93, 325-332.

Yang, H., Liu, W., Leng, Q., Hren, M.T., Pagani, M., 2011. Variation in *n*-alkane δD values from terrestrial plants at high latitude: Implications for paleoclimate reconstruction. Organic Geochemistry 42, 283-288.

Zech, M., Glaser, B., 2009. Compound-specific δ^{18}O analyses of neutral sugars in soils using gas chromatography-pyrolysis-isotope ratio mass spectrometry: problems, possible solutions and a first application. Rapid Communications in Mass Spectrometry 23, 3522-3532.

Zech, M., Pedentchouk, N., Buggle, B., Leiber, K., Kalbitz, K., Markovic, S.B., Glaser, B., 2011a. Effect of leaf litter degradation and seasonality on D/H isotope ratios of *n*-alkane biomarkers. Geochimica et Cosmochimica Acta 75, 4917-4928.

Zech, M., Zech, R., Buggle, B., and Zöller, L., 2011b. Novel methodological approaches in loess research – interrogating biomarkers and compound-specific stable isotopes. Eiszeitalter & Gegenwart – Quaternary Science Journal 60 (1), 170-187.

Zech, M., Werner, R.A., Juchelka, D., Kalbitz, K., Buggle, B., Glaser, B., 2012. Absence of oxygen isotope fractionation/exchange of (hemi-) cellulose derived sugars during litter decomposition. Organic Geochemistry 42, 1470-1475.

Zech, M., Tuthorn, M., Detsch, F., Rozanski, K., Zech, R., Zoeller, L., Zech, W., Glaser, B., 2013~~a~~. A 220 ka terrestrial δ^{18}O and deuterium excess biomarker record from an eolian permafrost paleosol sequence, NE-Siberia. Chemical Geology 360-361, 220-230.

~~Zech, M., Tuthorn, M., Glaser, B., Amelung, W., Huwe, B., Zech, W., Zöller, L., Löffler, J., 2013b. Natural abundance of 18O of sugar biomarkers in topsoils along a climate transect over the Central Scandinavian Mountains, Norway. Journal of Plant Nutrition and Soil Science 176, 12-15.~~

Zech, M., Mayr, C., Tuthorn, M., Leiber-Sauheitl, K., Glaser, B., 2014a. Oxygen isotope ratios (18O/16O) of hemicellulose-derived sugar biomarkers in plants, soils and sediments as paleoclimate proxy I: Insight from a climate chamber experiment. Geochimica et Cosmochimica Acta 126, 614-623.

Zech, M., Mayr, C., Tuthorn, M., Leiber-Sauheitl, K. and Glaser, B., 2014b. Reply to the comment of Sternberg on "Zech et al. (2014) Oxygen isotope ratios (^{18}O/^{16}O) of hemicellulose-derived sugar biomarkers in plants, soils and sediments as paleoclimate proxy I: Insight from a climate chamber experiment". GCA 126, 614-623. Geochimica et Cosmochimica Acta 141, 680-682.

Zech, M., Zech, R., Rozanski, K., Gleixner, G. and Zech, W., 2015. Do *n*-alkane biomarkers in soils/sediments reflect the δ^2H isotopic composition of precipitation? A case study from Mt. Kilimanjaro and implications for paleoaltimetry and paleoclimate research. Isotope in Environmental and Health Studies, accepted.

Formatiert: Schriftart: Kursiv

Formatiert: Schriftart: Kursiv

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Formatiert: Schriftart: Kursiv

Zech, R., Zech, M., Markovic, S., Hambach, U., Huang, Y., 2013~~c~~. Humid glacials, arid interglacials? Critical thoughts on pedogenesis and paleoclimate based on multi-proxy analyses of the loess-paleosol sequence Crvenka, Northern Serbia. Palaeogeography, Palaeoclimatology, Palaeoecology 387, 165-175.

List of Tables and Figures

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).

Fig. 1: Sampling ~~localities~~ 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.

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

Fig. 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}) pattern 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 site). Additionally, ~~A~~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~~is shown.

Fig. 4: Results of $\delta^2H_{\text{leaf water}}$ model simulations and comparison with ~~measured~~ biomarker-based ‘reconstructed’ (assuming a biosynthetic fractionation factor of -160 ‰) isotopic composition of leaf water ~~$\delta^2H_{n\text{-alkanes}}$ and $\delta^2H_{\text{fatty acids}}$~~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.

Fig. 5: δ^{18}O-δ^2H diagram illustrating the conceptual model of the coupled $\delta^2H_{n\text{-alkane}}$-$\delta^{18}O_{sugar}$ approach (modified after Zech M. et al., 2013a). ~~representing the global meteoric~~

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~~water line (GMWL) and an evaporation line (EL). Data for the~~ δ^2H~~δ^{18}O~~$_{n\text{-alkane}}$ (mean of n-C$_{29}$ and n-C$_{31}$) and δ^{18}O$_{sugar}$ (mean of arabinose, fucose and xylose) results are ~~values of hemicellulose-derived sugars (mean of arabinose, fucose and xylose) and the mean δ^2H values of leaf wax-derived *n*-alkanes (mean of *n*-C$_{29}$ and *n*-C$_{31}$) are displayed. δ^2H and δ^{18}O values of leaf water are reconstructed~~used to reconstruct δ^2H/δ^{18}O$_{leaf\ water}$~~-~~ by subtracting the ~~using~~ biosynthetic fractionation factors. ~~and the distance of reconstructed leaf water to the GMWL defines t~~The deuterium excess (d = δ^2H - 8·δ^{18}O) of leaf water serves as proxy for RH and ~~.~~ δ^2H~~and~~/δ^{18}O$_{prec}$ ~~values of precipitation for each site are~~is calculated as intersection of the individual evaporation lines (ELs, ~~(~~slope 2.82) with the global meteoric water line (GMWL).

Fig. 6: Comparison of ~~reconstructed humidity~~biomarker-based ~~on~~ ‘reconstructed’ relative humidity (RH) values ~~a normalized Craig-Gordon model accounting for deuterium excess and temperature~~ with ~~modern~~ actual ~~humidity data~~RH values (mean annual RH retrieved for all~~the~~ investigated sites ~~(~~from GeoINTA, 2012; summer daytime RH for six stations retrieved from www.ncds.noaa.gov). Deuterium excess values were calculated using δ^{18}O$_{leaf\ water}$ reconstructed from terrestrial sugars (Tuthorn et al., 2014) and δ^2H$_{leaf\ water}$ reconstructed from *n*-alkanes.

Fig. 7: Correlation of biomarker-based ‘reconstructed’ δ^{18}O$_{prec}$ and δ^2H$_{prec}$ values ~~reconstructed from the biomarkers~~ with ~~actual~~ modern ‘actual’ δ^{18}O$_{prec}$ and δ^2H$_{prec}$ values (from Bowen, 2012)~~, a and b, respectively~~..

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