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Halle, 3<sup>rd</sup> 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 $\delta^2$ 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")

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  $\delta^2 H$  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 \_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  $\delta^2 H_{n-alkane}$  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  $\delta^2 H_{n-alkane} - \delta^{18}O_{sugar}$  model is not based on the 20 samples analysed in this study for  $\delta^2 H_{n-alkane}$  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  $\delta^2 H_{n-alkane} - \delta^{18}O_{sugar}$  model.

 $\rightarrow$  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  $\delta^2 H_{n-alkane} - \delta^{18}O_{sugar}$  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  $\delta^2 H_{n-alkane} - \delta^{18}O_{sugar}$  model.

 $\rightarrow$  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 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  $\delta^2$ Hleaf water and  $\delta^{18}$ Oleaf 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  $\delta^2 H_{fatty acids}$  (weighted mean of  $n-C_{22}$ ,  $n-C_{24}$ ,  $n-C_{26}$ ,  $n-C_{28}$  and  $n-C_{30}$ ) pattern with  $\delta^2 H_{prec}$  (Bowen, 2012) along the north-south climate transect (<sup>x</sup>min and <sup>+</sup>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<sub>*n*-alkane- $\delta^{18}$ O<sub>sugar</sub> approach (modified after Zech M. et al., 2013a).  $\delta^{2}$ H<sub>*n*-alkane</sub> (mean of *n*-C<sub>29</sub> and *n*-C<sub>31</sub>) and  $\delta^{18}$ O<sub>sugar</sub> (mean of arabinose, fucose and xylose) results are used to reconstruct  $\delta^{2}$ H/ $\delta^{18}$ O<sub>leaf water</sub> 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<sub>prec</sub> is calculated as intersection of the individual evaporation lines (ELs, slope 2.82) with the global meteoric water line (GMWL)."</sub>

Furthermore, we changed the symbols (both in the graph and the legend) and rewrote chapter 2.4. Conceptual model for a coupled  $\delta^2 H \cdot \delta^{18} O$  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'  $\delta^2 H/\delta^{18}O_{prec}$  with biomarker-based 'reconstructed'  $\delta^2 H/\delta^{18}O_{prec}$ .

# *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.,  $2013 \rightarrow a$ , b, or c?

• *P.* 2472, line 3 : Tipple et al.  $\rightarrow$  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 $\rightarrow$ 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  $\rightarrow$  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,

Mano Tuthorn Michael Zech

Mario Tuthorn

&

& Michael Zech

Co-authors

Attachment: revised manuscript with track changes

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7			Formatiert: Englisch (USA)
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### 23 Abstract

24

25	The $\delta^2$ H isotopic composition of leaf waxes, especially of <u><i>p</i>-alkanes</u> ( $\delta^2$ H <sub><i>n</i>-alkanes</sub> ) is used		Formatiert: Schriftart: Kursiv	_
26	increasingly for paleohydrological and -climate reconstructions. However, it is challenging to			
27	disentangle past changes in the isotopic composition of precipitation and changes in			
28	evapotranspirative enrichment of leaf water. In order to overcome this limitation, Zech M. et			
29	al. (2013, Chemical Geology 360-361, pp. 220-230) proposed a coupled $\delta^2 H_{\pi-alkane} - \delta^{18} O_{sugar}$			
30	biomarker approach. This coupling allows We analyzed $\partial^2 H$ on <i>n</i> -alkanes and fatty acids in			
31	topsoils along a climate transect in Argentina, for which we had previously measured $\delta^{48}$ O on			
32	plant derived sugars. Our results indicate that leaf wax biomarker $\delta^2 H$ values ( $\delta^2 H_{lipids}$ )			
33	primarily reflect $\delta^2 H_{source-water}$ (precipitation), but are modulated by evapotranspirative			
34	enrichment. A mechanistic model is able to produce the main trends in $\delta^2 H_{lipids}$ along the			
35	transect, but seems to slightly underestimate evapotranspirative enrichment in arid regions			
36	and overestimate it in grass-dominated ecosystems. Furthermore, the (i) coupling of the			
37	$\partial^2 H_{lipid}$ and $\partial^{48} O_{sugar}$ biomarker results and (ii) application of biosynthetic fractionation factors			
38	allows-calculating calculating biomarker-based/-reconstructed the (i) $\delta^2 H - \frac{1}{\delta^{18} O_{\text{leaf water}}}$ values,			
39	isotopic composition of leaf water along the transect. This also yields(ii) the deuterium excess			
40	(d-excess) of leaf water, which mainly reflects evapotranspirative enrichment, and can be			
41	used to model-reconstruct relative air humidity (RH) and (iii) $\delta^2 H/\delta^{18} O_{\text{precipitation}}$ , values. Here			
42	were present a respective climate transect validation study by coupling new results from $\delta^2 H$			
43	analyses on <i>p</i> -alkanes and fatty acids in topsoils along a climate transect in Argentina with		Formatiert: Schriftart: Kursiv	_
44	previously measured $\delta^{18}$ O results obtained for plant-derived sugars. Accordingly, both the			
45	<u>Correlating the reconstructed RH and <math>\delta^2 H/\delta^{18} O_{\text{precipitation}}</math> values correlate highly significantly</u>	(	Formatiert: Englisch (Großbritannien)	_
46	with actual RH and $\delta^2 H/\delta^{18}O_{\text{precipitation}}$ values. We conclude that compared to single $\delta^2 H_{lkalkane}$		Formatiert: Schriftart: Kursiv, Tiefgestellt	
47	or $\delta^{18}O_{sugar}$ records, the proposed coupled $\delta^2H_{\pi-alkane}-\delta^{18}O_{sugar}$ approach will allow more robust		Formatiert: Tiefgestellt Formatiert: Tiefgestellt	_
48	to the measured RH, as well as the reconstructed and actual isotopic composition of		Formatiert: Englisch (Großbritannien)	

49	precipitation, we tested the value of combined $\delta^2 H$ and $\delta^{18} O$ approach for the reconstruction	 Formatiert: Englisch (Großbritannien)
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50	$\underline{\text{of}} \partial^2 H/\partial^{18} O_{\text{precipitation}}$ the isotopic signal of past precipitation reconstructions and additionally	 Formatiert: Englisch (Großbritannien)
51	the reconstruction of RH changes/history in future - We conclude that the good agreement and	 Formatiert: Englisch (Großbritannien)
52	high correlation between the values lend support to using the combined $\delta^2 H$ and $\delta^{48} \Theta$	 Formatiert: Englisch (Großbritannien)
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53	measurement of lipid and sugar biomarkers for paleoclimate research The high correlation of	
55	measurement of high and sugar biomarkers for parcoerinate research, the high conclusion of	
54	modeled (reconstructed based on biomarker results) and measured RH, as well as the good	
55	agreement between modeled and actual $\delta^{2}$ H and $\delta^{18}$ O of precipitation along the transect lends	
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59	<b>Keywords:</b> paleoclimate proxies, nemicellulose sugars, <i>n</i> -alkanes, leaf water enrichment,	

60 deuterium-excess, relative air humidity

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#### 62 1. Introduction

63

64 Long chain *n*-alkanes and fatty acids are important components of the epicuticular leaf waxes 65 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 66 67 serve as valuable biomarkers for paleo-environmental and -climate reconstructions (Eglinton and Eglinton, 2008; Zech M. et al., 2011b). The  $\delta^2$ H isotopic composition of leaf waxes is of 68 69 particular interest in this regard, because, at least to a first order, it reflects the isotopic 70 composition of precipitation <u> $\delta^2 H_{prec}$ </u> (Sauer et al., 2001; <u>Huang et al., 2004</u>; Sachse et al., 71 2004; Schefuss et al., 2005; Pagani et al., 2006; Tierney et al., 2008; Rao et al., 2009), which 72 in turn depends on temperature, humidityamount of precipitation, atmospheric circulation, etc. (Dansgaard, 1964; Rozanski et al., 1993; Gat, 1996; Araguas-Araguas et al., 2000). While 73 74 there is probably no fractionation of hydrogen isotopes during water uptake by the roots 75 (Ehleringer and Dawson, 1992), several studies have shown that leaf water is enriched in <sup>2</sup>H compared to the source water or precipitation (Flanagan et al., 1991; Yakir, 1992; Sachse et 76 77 al., 2006; Smith & Freeman, 2006; Farquhar et al., 2007; Feakins & Sessions, 2010). This <sup>2</sup>H enrichment, which is also recorded in the leaf waxes, can be explained by evapotranspiration 78 79 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 80 81 evapotranspirative enrichment in the past can make it challenging to reconstruct the isotopic composition of paleoprecipitation from  $\delta^2$ H biomarker records alone. 82 Compound-specific  $\delta^2 H$  of lipid biomarkers, especially *n* alkanes due to their good 83 preservation, are already widely applied in paleoclimate and hydrological research (Sauer et 84 85 al., 2001; Schefuss et al., 2005; Pagani et al., 2006; Tierney et al., 2008; Zech et al., 2013c). a 86 robust reconstruction of  $\delta^2 H_{\text{prec}}$  from soils and sedimentary records turns increasingly out to

87 <u>be quite challenging, because it is hitherto difficult to disentangle past changes in  $\delta^2 H_{\text{prec}}$  and</u>

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88	changes in evapotranspirative enrichment of leaf water (Zech, R. et al., 2013; Zech, M. et al.,
89	2015). Comparison of $\delta^2 H_{n-alkanes}$ with $\delta^2 H_{fatty-acids}$ in such research is hardly done so far but
90	may provide additional merits of using fatty acids as an alternative to <i>n</i> -alkanes. Similarly
91	<u>Compared to compound-specific <math>\delta^2</math>H analyses</u> , compound-specific $\delta^{18}$ O analyses of sugars
92	remain in their infancy, yet hold significant promiseare by far less adopted by the scientific
93	community, so far (Hener et al., 1998; Juchelka et al., 1998; Werner, 2003; Jung et al., 2005;
94	Jung et al., 2007; Greule et al., 2008). <u>However, particularly compound-specific <math>\delta^{18}</math>O analyses</u>
95	of hemicellulose-derived sugar biomarkers ( $\delta^{18}O_{sugars}$ ) extracted from plants, soils and
96	sediments are proposed to have large potential especially in paleoclimate/-hydrologic
97	research: (Zech M. & Glaser, 2009; Zech M. et al., 2012). Similar to leaf waxes,
98	hemicellulose-derived sugars record the isotopic composition of water used for metabolism,
99	i.e. the isotopic composition of precipitation altered by evapotranspirative <sup>18</sup> O enrichment of
100	soil and leaf water (Zech et al., 2013bTuthorn et al., 2014; Zech M. et al., 2014a).
101	Hemicellulose residues can persist in soils (Zech et al., 2012)Recently, Zech M. et al. (2013)
102	proposed a conceptual coupled $\delta^2 H_{\eta-alkane} - \delta^{18} O_{sugar}$ model for paleoclimate research and
103	suggested that this coupling allows overcoming the above defined limitation of single $\delta^2 H_{n-1}$
104	alkane approaches. Accordingly, the coupled $\delta^2 H_{\eta$ -alkane $\delta^{18}O_{sugar}$ approach allows reconstructing
105	(i) $\delta^2 H/\delta^{18}O_{\text{leaf water}}$ values, (ii) deuterium excess (d-excess) of leaf water, which mainly
106	reflects evapotranspirative enrichment and can be used to reconstruct relative air humidity
107	(RH) and (iii) $\delta^2 H/\delta^{18}O_{prec}$ values. Given the evaporative <sup>2</sup> H and <sup>48</sup> O enrichment of leaf water
108	being recorded in both, a alkanes and hemicellulose derived sugars the combined plant wax
109	$\delta^2$ H and hemicellulose sugar $\delta^{18}$ O analyses offer the potential of reconstructing the deuterium
110	excess of leaf water. The d-excess quantifies the isotopic deviation of water from the Global
111	Meteoric Water Line (GMWL) and may serve as valuable proxy for evapotranspirative
112	enrichment and RH. Furthermore, if leaf water values are defined in a $\delta^2$ H $\delta^{48}$ O diagram,

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113	sugar and <i>n</i> alkane isotopic information may enable reconstruction of the isotopic	Formatiert: Schriftart: Kursiv
114	composition of precipitation in paleohydrological studies (Zech et al., 2013a).	
115	Theis here presented study describes the application of a combined aimed at validating or	
116	<u>falsifying the coupled</u> $\delta^2 H$ and $\delta^{18} O$ biomarker approach by applying it to a modern topsoils	
117	sampled along a climate transect fromin Argentina. The aims of this investigation are toMore	
118	specifically, we aimed at (i)	
119	(i)-analysing and compareing the $\delta^2$ H values of <i>n</i> -alkanes with those of and fatty acids, (ii)	
120	modellinged <i>n</i> -alkane-leaf water enrichment along the transect and comparison with $\delta^2 H_{\mu}$	Formatiert: Tiefgestellt
121	$alkanes$ and $\delta^2 H_{fatty acids}$ values and $\delta^{48}$ O values of sugars and evaluate the dominant climate	
122	factors influencing these values,	
123	(iii) reconstructing d-excess of leaf water using the coupled $\delta^2 H_{\underline{n_ralkane}}$ values of <i>n</i> alkanes	Formatiert: Schriftart: Kursiv, Tiefgestellt
124	and $\delta^{18}O$ values of super approach and assess evaluating the potential of for reconstructinged	Formatiert: Tiefgestellt
125	d excess of leaf water as paleoclimate proxy for RH, and	
126	(ivii) reconstructing 'biomarker-based' $\delta^2 H/\delta^{18}O_{prec}$ values and evaluate the potential of the	
127	combined $\delta^{18}O$ and $\delta^2H$ biomarker approach to reconstruct th <u>comparison with actual</u>	
128	$\delta^{2}$ H/ $\delta^{18}$ O <sub>prec</sub> values.e isotopic composition of precipitation.	
129		
130	2. Material and methods	

131

### 132 **2.1. Transect description and samples**

133 The investigated transect in Argentina spans from ~32°S to 47°S, and encompasses 20 134 sampling localities-locations spanning a large climate and altitudinal (22 – 964 m) gradient 135 (Fig. 1). Mean annual temperature ranges from 11.4 °C to 18.0 °C and mean annual 136 precipitation from 185 mm to 1100 mm (GeoINTA, 2012). Precipitation shows a systematic 137 southward trend towards more negative  $\delta^{18}$ O and  $\delta^{2}$ H values ( $\delta^{18}$ O<sub>prec</sub> and  $\delta^{2}$ H<sub>prec</sub>, 138 respectively) (Bowen, 2012; GeoINTA, 2012). 139 The transect is described in detail by Tuthorn et al. (2014) and Ruppenthal et al. (2015). 140 Briefly, it is characterized by warm humid subtropical conditions in the north (Zárate, Buenos 141 Aires Province), pronounced arid conditions in the middle part of the transect and cool 142 temperate conditions in the south (Las Heras, Santa Cruz Province). These markedly 143 contrasting climate conditions are reflected in the vegetation zones of the study area, changing 144 from Humid/Dry Pampa (with dominance of Triticum, Setaria, Eragrostis, Andopogon, 145 Panicum and Festuca species) in the north to the Espinal vegetation zone (with dominance of 146 Festuga and Larrea species) that prevails under semi-arid climate (Burgos and Vidal, 1951), 147 Low Monte semidesert/desert (with dominance of Larrea species) in the most arid region of 148 Argentina (Fernández and Busso, 1997), and Patagonian Steppe (with dominance of Stipa 149 species) in the southernmost part of the transect (Le Houérou, 1996; Paruelo et al., 1998). 150 During a field campaign in March and April 2010, mixed topsoil samples (Ah-horizons) from 151 maximum 51 cm depth were collected in triplicate replication from the 20 sample sites along 152 the transect (for soil type and total organic carbon contents please see Table 1 of Tuthorn et 153 al., 2014). The soil samples were air-dried in the field and later in an oven at 50°C for several 154 <u>days</u>. While The sampling site heterogeneity was checked for the  $\delta^{18}O_{sugar}$  analyses and in 155 most cases did not exceed the analytical uncertainty (Table 2 in Tuthorn et al., 2014). 156 Therefore, the field replications were merged to one composite sample per study site for 157  $\delta^2 H_{lipid}$  analyses.

158

### 159 **2.2. Compound-specific** $\delta^2$ **H** analyses of *n*-alkanes and fatty acids

For  $\delta^2$ H 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. (2013e). 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 displayed no requirement for this purification step.

172 Fatty acids were methylated using 5% HCl in methanol at 80°C for 12 hours. Subsequently, 173 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<sub>2</sub> columns (~2 g). 174 175  $5\alpha$  and rost and hexamethylben zene was used for quantification of the compounds on an 176 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-177 specific  $\delta^2$ H values of the long-chain *n*-alkanes and FAMEs were determined based on at least 178 179 triplicate analyses on a gas chromatograph-pyrolysis-isotope ratio mass spectrometer (GCpyrolysis-IRMS, Delta V, ThermoFisher Scientific, Bremen, Germany). The A4 standard 180 181 mixture (provided by Arndt Schimmelmann, Indiana University, USA) was run three times 182 per sequence at three different concentrations. All results are reported after normalization 183 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 184 precision of the analyses was monitored using a laboratory standard (oak,  $n-C_{29}$ ). The 185 standard was analyzed in every sequence and yielded a mean value of -147.2‰ with a 186 187 standard deviation of  $\pm 1.7$  ‰ across all sequences run for this study.

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### 189 2.3. Modeling of leaf water <sup>2</sup>H enrichment

190 The empirical data analyses were combined with mechanistic model simulations of  $\delta^2 H_{\text{leaf water}}$  in order 191 to better detect and evaluate how the dominant climate variables (air temperature and relative air 192 humidity) influence <sup>2</sup>H enrichment in lipids. The\_<sup>2</sup>H enrichment of leaf water due to 193 evapotranspiration can be predicted by using mechanistic models originally developed for 194 isotope fractionation processes associated with evaporation from water surfaces by Craig and 195 Gordon (1965). These models were adapted for plants by Dongmann et al. (1974) and subsequently Farquhar and Lloyd (1993). Evaporative <sup>2</sup>H enrichment of the leaf water ( $\Delta^2 H_e$ ) 196 197 at the evaporative surface in the mesophyll is given by the equation:

198 
$$\Delta^2 H_e = \varepsilon^+ + \varepsilon_k + \left(\Delta^2 H_{WV} - \varepsilon_k\right) \frac{e_a}{e_i}, \qquad (\text{Eqn. 1})$$

199 where  $\varepsilon^+$  is the equilibrium fractionation between liquid water and vapor at the air-water 200 interfaces (Bottinga and Craig, 1969),  $\varepsilon_k$  is the kinetic fractionation during water vapor diffusion from leaf intercellular air space to the atmosphere,  $\Delta^2 H_{WV}$  is the isotopic difference 201 202 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 203 204 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 205 evaporation (Farquhar and Lloyd, 1993): 206

207 
$$\Delta^2 H_{leafwater} = \frac{\Delta^2 H_e \left(1 - e^{-\varphi}\right)}{EL/CD}.$$
 (Eqn. 2)

The quotient of EL/CD represents the Péclet number ( $\delta^2$ ) 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  $^{12}H_2^2H_2O$ . 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  $\delta^2$ H. This simplified model is based on the assumption that leaf temperature equals air temperature and that atmospheric vapor  $\delta^2$ H is in

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214	equilibrium with source water $\delta^2 H$ (Kahmen et al. 2011b). Transpiration rates are estimated	
215	using relative humidity and air temperature (retrieved from GeoINTA, 2012) and assuming a	
216	mean stomatal conductance of 0.15 mol/m <sup>2</sup> /s. Based on reports for a large number of species	
217	in the literature (Kahmen et al., 2008; Kahmen et al., 2009; Song et al., 2013), we used an	
218	average value of 20 mm for L and kept it constant across the transect. For our simulation of	
219	leaf water $\delta^2 H$ values we obtained the model input variables air temperature, atmospheric	
220	vapor pressure and source water $\delta^2$ H from GeoINTA (2012) and Bowen (2012), respectively.	
221	The isotopic composition of the leaf water can be estimated according to Eqn. 3:	
222		
223	$\delta^{2} H_{\text{leaf water}} = \Delta^{2} H_{\text{leaf water}} + \delta^{2} H_{\text{SW}} $ (Eqn.3),	
224		
225	where $\Delta^2 H_{\text{leaf water}}$ is the bulk leaf water evaporative enrichment and $\delta^2 H_{\text{SW}}$ is the hydrogen	
226	isotope ratio of source/xylem water.	
227		
228	2.4. The e <u>C</u> onceptual model for a coupled $\delta^{18}$ O- $\delta^2$ H <u>biomarker</u> approach	
229	The conceptual coupled $\delta^2 H_{\eta_c alkane} \delta^{18} O_{sugar}$ model was introduced previously by Zech M. et al.	Formatiert: Schriftart: Nicht Fett
230	(2013). In brief, it is based on the following fundamentals. Precipitation word-wide typically	Formatiert: Schriftart: Kursiv, Tiefgestellt Formatiert: Tiefgestellt
231	plots along/close to the so-called global meteoric water line (GMWL, $\delta^2 H = 8 \times \delta^{18} O + 10$ ) in	Formatiert: Tiefgestellt
2.51		Formatiert: Schriftart: Times New Roman, 12 Pt. Formatiert: Schriftart: Times New Roman, 12 Pt.
232	<u>a</u> $\partial^{10}$ O- $\partial^2$ H diagram (Dansgaard, 1964) (Fig. 5), Due to fractionation processes,	Formatiert: Schriftart: Times New Roman, 12 Pt.
233	evaporation/transpiration causes evaporating water vapour to be isotopically depleted in <sup>18</sup> O	Formatiert: Schriftart: Times New Roman, 12 Pt.
234	and <sup>2</sup> H, whereas residual (leaf) water ( $\delta^2 H/\delta^{18}O_{\text{leaf water}}$ ) is isotopically enriched. In a $\delta^{18}O-\delta^2 H$	Formatiert: Schriftart: Times New Roman, 12 Pt.
235	diagram, leaf water therefore does not plot on the GMWL but on an evaporation line (EL).	Formatiert: Schriftart: Times New Roman, 12 Pt.
236	€The distance of reconstructed leaf water to the Global Meteoric Water Line (GMWL) can be	
237	de <u>scribed</u> fines the as deuterium excess (d = $\delta^2 H - 8 \cdot \delta^{18} O$ ). Observed deviation is caused by	
238	the kinetic effect resulting in slower diffusivity of the <sup>4</sup> H <sup>4</sup> H <sup>48</sup> O molecules compared to the	
239	<sup>2</sup> H <sup>4</sup> H <sup>46</sup> O moleculesMore 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., 2013e):

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

245 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 246 25.1 ‰ and 28.5 ‰ for  $C_k^2$  and  $C_k^{18}$ , respectively, considering that these are the maximum 247 248 values of kinetic fractionation during molecular diffusion of water through stagnant air. 249 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 *p*-alkanes 250 251 and sugars in plants and soils reflect (albeit with a constant offset caused by biosynthetic 252 fractionation) the isotopic composition of leaf water, a coupled  $\delta^2 H_{\eta$ -alkane- $\delta^{18}O_{sugar}$  approach 253 allows reconstructing RH values. 254 The combined  $\delta^{48}O$   $\delta^{2}H$  biomarker. The biomarker-based reconstructed  $\delta^{2}H/\delta^{18}O_{\text{leaf water}}$ 255 values approach also allows furthermore reconstructioning of the isotopic composition of plant 256 source water, which can be considered as in an approximation to reflect for  $\delta^2 H_{prec}$  and 257  $\delta^{18}O_{\text{prec}}$  (illustrated In Figure 5 these are given by the sintercepts 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- $\delta^{2}$ H biomarker results the reader is referred to Zech M. et al. (2013a).

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#### 266 3. Results and Discussion

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### 268 **3.1. Comparison of** $\delta^2$ **H***n*-alkanes and $\delta^2$ **H**fatty acids

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

275 The  $\delta^2$ H values of the fatty acids *n*-C<sub>22</sub>, *n*-C<sub>24</sub>, *n*-C<sub>26</sub>, *n*-C<sub>28</sub> and *n*-C<sub>30</sub> range from -128 to -225 276 % (Table 1 and Fig. 2). In general, there is a good overall agreement between the *n*-alkanes 277 and the fatty acids (R=0.96, p<0.001, n=20; for the weighted means), both showing much 278 more negative  $\delta^2 H$  values in the south than in the northern and middle portions of the transect 279 (Table 1, Fig. 2). Interestingly, the longer homologues n-C<sub>28</sub> and n-C<sub>30</sub> are systematically 280 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 <sup>2</sup>H relative to the corresponding 281 282 *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 283 284 homologue sources. Roots, for example, have also been suggested as a source of long-chain n-285 fatty acids (Bull et al., 2000). Shorter homologues, have been suggested to be not only plantderived, but also of bacterial origin (Matsumoto et al., 2007; Bianchi and Canuel, 2011). 286 287 Similarly, soil microbial overprinting of long chain *n*-alkanes and fatty acids cannot be 288 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 289

<u>Kahmen, 2015</u>) and not significantly introduced into soils/subsoils by roots (Häggi et al., 2014).

The consistent  $\delta^2$ H pattern revealed by the *n*-alkanes and fatty acids along the north-south climate transect does not solely reflect the  $\delta^2$ H isotopic composition of precipitation. Especially in the middle part of the transect,  $\delta^2$ H 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  $\delta^2$ H of long chain *n*-alkanes in further discussion and calculations.

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### 299 **3.2. Evapotranspirative <sup>2</sup>H enrichment of leaf water**

300 Assuming a consistent constant biosynthetic fractionation of  $-160 \ \%$  for the *n*-alkane and 301 fatty acids biosynthesis in plants (Sessions et al., 1999; Sachse et al., 2006), we estimated the 302 isotopic composition of leaf water using our *n*-alkane and fatty acids  $\delta^2 H$  values along the 303 transect/gradient (Fig. 3). Note that an average biosynthetic fractionation factor of ~-200 ‰ 304 was reported by Sessions et al. (1999) for short- and mid-chained fatty acids synthesized 305 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  $\delta^2 H$ 306 307 *n*-alkanes and fatty acids values are very similar, using a biosynthetic fractionation factor of -308 160 ‰ for both lipids seems appropriate.

Estimated leaf water  $\delta^2$ H values suggest a pronounced <sup>2</sup>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  $\delta^2 H_{\text{leaf water}}$  values inferred from the measured *n*-alkanes and fatty acids, and  $\delta^2 H_{\text{leaf water}}$  values calculated using the Peclet-

316 modified Graig-Gordon model. The correlations are highly significant (r=0.88, p<0.001, 317 n=20, for *n*-alkanes and r=0.93, p<0.001, n=20 for fatty acids), suggesting that the model 318 correctly implements the most relevant processes related to evapotranspirative enrichment of 319 leaf water. While predicting the overall trend in leaf water  $\delta^2 H$  along the transect with 320 reasonable accuracy, the model does not capture site-to-site excursions in the n-alkane-321 derived leaf water  $\delta^2 H$  values from this overall trend. As such, additional influences that are 322 not captured by the model, such as evaporative <sup>2</sup>H enrichment of soil water, can possibly could 323 explain the underestimation of the modeled  $\delta^2 H_{\text{leaf water}}$  values in the middle part of the transect 324 (Fig. 4). In contrast, the model might overestimate  $\delta^2 H_{\text{leaf water}}$  in the northern and the southern part of the transect. The corresponding ecosystems, the Humid Pampa and the Patagonian 325 326 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 327 328 leaf water <sup>2</sup>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 329 may be due to plant species effects (Pedentchouk et al., 2008; Douglas et al., 2012). The more 330 331 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. 332

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 ~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  $\delta^{18}$ O values of hemicellulose-derived arabinose, fucose and xylose analysed in topsoils along the investigated transect (Tuthorn et al., 2014). Model sensitivity tests of <sup>18</sup>O enrichment of leaf water using 342 PMCG model corroborate the observations presented here that air humidity is the key factor

- 343 defining the <sup>18</sup>O/<sup>2</sup>H enrichment of leaf water.
- 344

#### 345 3.3. Combining Coupling of the $\delta^2 H_{n-alkane}$ and $\delta^{18}O_{sugar}$ and $\delta^2 H_{n-alkane}$ biomarker Formatiert: Tiefgestellt Formatiert: Tiefgestellt 346 analyses results 347 A-The conceptual model for the combined interpretation of $\delta^2 H_{n-alkane}$ and $\delta^{18}O_{sugar}$ biomarker approaches can beis illustrated in a $\delta^{18}$ O $\delta^{2}$ H diagram (Fig. 5). The model is based 348 349 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 350 transect investigated here, this assumption is reasonable and supported by leaf water modeling 351 352 (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' $\delta^2 H/\delta^{18} O_{\text{leaf water}}$ values can 353 Formatiert: Tiefgestellt 354 be calculated from the isotopic composition of the biomarkers by applying biosynthetic 355 fractionation factors Ebio. an average value according to various studies of the biosynthetic Formatiert: Tiefgestellt 356 fractionation factors resulting inFor our reconstructions we applied Ebio factors of -160 ‰ 357 (Sessions et al., 1999; Sachse et al., 2006) and +27 ‰ (Sternberg et al., 1986; Yakir and 358 DeNiro, 1990; Schmidt et al., 2001; Cernusak et al., 2003; Gessler et al., 2009) for $\delta^2$ H and 359 $\delta^{18}$ O, respectively (Fig. 5). 360 361 3.3.1. Reconstructed RH values along the climate transect and comparison with actual

362 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-Gordoncoupled  $\delta_{2}^{2}H_{n-alkane} - \delta_{2}^{18}O_{sugar}$  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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368 systematic d-excess trend and correlate significantly (r=0.79, p<0.001, n=20) with the actual 369 modern-mean annual RH values retrieved from GeoINTA (2012) for all investigated sites.<sup>5</sup>

which generally validates the  $\delta^{18}$ O- $\delta^{2}$ H conceptual model. 370 371 However, as depicted by Fig. 6, the reconstructed RH values systematically underestimate the 372 actual (modern)mean annual RH values. This is especially pronounced for the three 373 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  $\delta^{18}O$ - $\delta^{2}H$ 374 375 diagram, the soil water enrichment shifts the source water (simplified to 'reconstructed 376 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 377 378 water lies on the GMWL in the model, while local meteoric water lines and thus actual 379 precipitation may have a d excess offset from the GMWL (d-excess of GMWL = 10 ‰). In 380 our case, this effect should be negligible, as d excess values of precipitation along the transect 381 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; 382 383 Riederer & Markstaedter, 1996; Kahmen et al., 2011a; Tipple et al., 20132) they may not necessarily reflect the mean annual isotopic composition of precipitation in regions with 384 385 pronounced seasonality, but rather the isotopic composition of precipitation during the growing season. As wellFurthermore, compared to themean annual (modern) RH values likely 386 387 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 388 389 especially pronouncedIndeed 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 <sup>2</sup>H enrichment

394 of leaf water, which is the case for grasses (section 3.2; McInerney et al., 2011; Kahmen et al., 395 2013b). In the  $\delta^{18}$ O  $\delta^{2}$ H diagram, leaf water would thus plot lower than the simple Craig-396 Gordon model predicts, and d-excess would be too negative. 397 ThirdFinally, the  $\delta^{18}$ O biosynthetic fractionation factor of ~+27 ‰, which has been reported 398 for <u>newly assimilated sugars and cellulose</u>, may underestimates in our opinion the actual 399 fractionation factor of hemicelluloses (Tuthorn et al., 2014; Zech M. et al., 2014a)., which 400 <u>This would</u> results in reconstructed leaf water values plotting too far to the right in the  $\delta^{18}$ O-401  $\delta^2$ H diagram (Fig. 5) and in turn to the observed underestimated RH values (Fig. 6). This can 402 be explained-We argue with the loss of a relatively <sup>18</sup>O-depleted oxygen atom attached to C-6 403 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 404 405 80% of the oxygen atoms at the C 6 position are isotopically exchanged during cellulose 406 synthesispoint to a recent study of (Waterhouse et al., (2013) who have determined the 407 position specific  $\delta^{18}$ O values in cellulose. Further experimental studies as suggested and 408 encouraged by Sternberg (2014) and Zech M. et al. (2014b) are urgently needed to ascertain 409 an improved biosynthetic fractionation factor for hemicellulose-derived sugars. In contrast, 410 the value of +27 % would be an overestimation in cases where significant amounts of stem or 411 root derived sugars contribute to the soil sugar pool, because up to 40% of the oxygen atoms 412 being biosynthesized in leaves are exchanging with non-enriched root/stem water during 413 cellulose biosynthesis in roots/stems (Sternberg et al., 1986; Gessler et al., 2009). However, 414 given that thissuch an exchange would result in an overestimation of reconstructed RH values 415 (the opposite is observed, Fig. 6), we suggest that the majority of the sugar biomarkers along 416 the topsoil transect investigated here are leaf-derived and not stem /root-derived. 417

418 3.3.2. Comparison of reconstructed and actual  $\delta^2 H_{prec}$  and  $\delta^{18}O_{prec}$  values

419 Values of  $\delta^{18}O_{prec}$  and  $\delta^{2}H_{prec}$  reconstructed as the intercepts of the individual evaporation 420 lines (EL) with the GMWL in the  $\delta^{18}$ O- $\delta^{2}$ H diagram (Fig. 5) range from -7 to -22 ‰ and from 421 -47 to -166 ‰, respectively. They correlate highly significantly (Fig. 7; r=0.90, p<0.001, 422 n=20, and r=0.88, p<0.001, n=20 for  $\delta^{18}O_{prec}$  and  $\delta^{2}H_{prec}$ , respectively) with the <u>\_actual</u>  $\delta^2 H_{prec}$  and  $\delta^{18}O_{prec}$  values as derived from Bowen (2012). While the reconstructed  $\delta^{18}O_{prec}$ 423 424 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 425 426 precipitation water (Fig. 7).

427 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 428 429 actual values for the isotopic composition of precipitation as Hence, the 'actual'  $\delta^2 H / \delta^{18} O_{\text{prec}}$ 430 values used for our comparison with the biomarker-based 'reconstructed values can be 431 assumed to be one the uncertainty factor. reported by While Bowen (2012) show-reported a 432 confidence interval (95%) ranging from 0.2% to 1.2%, and from 2% to 11% for  $\delta^2 H_{\text{prec}}$  and 433  $\delta^{18}O_{prec}$ , respectively, future climate transect studies will be ideally carried out with actual 434 precipitation being sampled for  $\delta^2 H/\delta^{18}O$  analyses. MoreoverHere, we would like to emphasize also here the possible very likely influence of seasonality. As reported for sugar 435 436 biomarkers (Tuthorn et al., 2014), we suggest that also leaf waxes as well likely mainly reflect 437 the humidity and the isotopic composition of spring and summer precipitation rather than 438 mean annual values. Accounting for these seasonality effects, the apparent offsets would be 439 reduced. Future modeling studies should therefore pay particular attention to seasonality, and 440 consider using climate parameters of the growing season instead of annual means.

441

#### 442 5. Conclusions

<sup>1</sup> 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  $\delta^{2}H_{\mu-alkane}-\delta^{18}O_{sugar}$  model and from Bowen's (2012) online isotopes in precipitation calculator.

Formatiert: Englisch (Großbritannien) Formatiert: Schriftart: 10 Pt. Formatiert: Schriftart: 10 Pt. Formatiert: Englisch (Großbritannien) 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  $\delta^2$ H values ranging from -155 to -222 ‰ and -128 to -225 ‰, respectively. These  $\delta^2$ H values broadly parallel variations in the hydrogen isotopic composition of precipitation, but are modulated by evaporative <sup>2</sup>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.

451 Based on the premise that *n*-alkanes and hemicellulose biomarkers are primarily leaf-derived, 452 we reconstruct  $\delta^2 H_{\text{leaf water}}$  and  $\delta^{18} O_{\text{leaf water}}$ , respectively, which in turn allows assessment of 453 the d-excess of leaf water. The large calculated range in d-excess along the transect (-67 to -454 178 ‰) can be used to calculate/model\_biomarker-based 'reconstructed' RH.; modeled 455 'Reconstructed' RH correlates significantly with empirical modernactual mean annual RH 456 along the transect. Despite this overall correlation, we observe a systematic underestimation 457 of <u>'reconstructed'</u> RH, which we attribute to limitations of the combined  $\delta^{18}O = \delta^{2}H_{is}$ . 458 however, largely reduced when comparing with actual summer daytime RH values conceptual model. Similarly, <u>biomarker-based</u> 'reconstructed'  $\delta^{18}O_{prec}$  and  $\delta^{2}H_{prec}$  calculated/modeled 459 460 from biomarker isotopic compositions significantly values correlate highly significantly with 461 modern 'actual' precipitation. 462 The novel combined We conclude that compared to single  $\delta^2 H_{n-alkane}$  or  $\delta^{18}O_{sugar}$  records, the

463 proposed coupled  $\delta^2 H_{n-alkane} - \delta^{18}O_{sugar}$  approach will allow more robust  $\delta^2 H/\delta^{18}O_{prec}$ 464 reconstructions and additionally the reconstruction of summer daytime RH changes/history 465 using d-excess of leaf water as proxy in future paleoclimate studies.  $\delta^2 H - \delta^{18}O$  biomarker 466 approach has great potential for paleo hydrological and paleo climate reconstructions. In 467 principle, it allows determination of d excess of past leaf water, thus constraining evaporative 468 enrichment, as well as the isotopic signal of past precipitation.

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469

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471

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735	List of Tables and Figures		
736			
750			
737	<b>Table 1:</b> $\delta^2$ H values of individual leaf wax <i>n</i> -alkanes and fatty acids. Measurements were		
738	carried out in at least triplicate (sd = standard deviation).		
739	Fig. 1: Sampling localities locations along the investigated transect in Argentina. The colors		
740	illustrate the gradient in $\delta^2 H_{prec}$ , and mean annual temperature and precipitation are		
741	shown below.		
742	Fig. 2: Comparison of $\delta^2 H$ results of individual leaf wax <i>n</i> -alkanes and <i>n</i> -alkanoic (fatty)		
743	acids along the investigated transect.		
744	Fig. 3: Comparison of measured $\delta^2 H_{n-alkanes}$ (weighted mean of $n-C_{29}$ and $n-C_{31}$ ) and $\delta^2 H_{fatty}$		
745	acids (weighted mean of <i>n</i> -C <sub>22</sub> , <i>n</i> -C <sub>24</sub> , <i>n</i> -C <sub>26</sub> , <i>n</i> -C <sub>28</sub> and <i>n</i> -C <sub>30</sub> ) pattern with $\delta^2 H_{prec}$		
746	(Bowen, 2012) along the north-south climate transect (xmin and xmax representing	$\leq$	Formatiert: Hochgestellt
747	annual minimum and maximum value at the sampling site). Additionally, Aassuming a	٦	Formatiert: Hochgestellt
748	consistent biosynthetic fractionation of -160 ‰ for the <i>n</i> -alkane and fatty acid		
749	biosynthesis in plants the biomarker-based 'reconstructed' isotopic composition of leaf		
750	water was inferredis shown.		
751	Fig. 4: Results of $\delta^2 H_{\text{leaf water}}$ model simulations and comparison with measured-biomarker-		
752	based 'reconstructed' (assuming a biosynthetic fractionation factor of -160 %)		
753	isotopic composition of leaf water $\delta^2 H_{p-alkanes}$ and $\delta^2 H_{fatty acids}$ based on <i>n</i> -alkanes and		Formatiert: Schriftart: Kursiv
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/54	Tatty actus, respectively. Sensitivity tests for o'meaf water are shown for changes in KH		Formatiert: Schriftart: Nicht Kursiv
755	and air temperature for all 20 sites along the transect.		
756	Fig. 5: $\delta^{18}$ O- $\delta^{2}$ H diagram <u>illustrating the conceptual model of the coupled <math>\delta^{2}_{2}</math>H<sub>n-alkane</sub>-<math>\delta^{18}</math>O<sub>sugar</sub></u>		Formatiert: Schriftart: Times New Roman, 12 Pt.
757	approach (modified after Zech M. et al., 2013a). <del>representing the global meteoric</del>	$\square$	Formatiert: Schriftart: Times New Roman, 12 Pt., Englisch (Großbritannien)
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758	water line (GMWL) and an evaporation line (EL). Data for the $\partial^2 H \partial^{18} \Theta_{n,alkane}$ (mean of	Formatiert: Schriftart: Kursiv, Tiefgestellt
759	<u><i>n</i>-C<sub>29</sub> and <i>n</i>-C<sub>31</sub>) and <math>\delta^{18}O_{sugar}</math> (mean of arabinose, fucose and xylose) results are values</u>	
760	of hemicellulose-derived sugars (mean of arabinose, fucose and xylose) and the mean	
761	$\delta^2$ H values of leaf wax-derived <i>n</i> alkanes (mean of <i>n</i> -C <sub>29</sub> and <i>n</i> -C <sub>31</sub> ) are displayed. $\delta^2$ H	
762	and $\delta^{18}O$ values of leaf water are reconstructed used to reconstruct $\delta^{2}H/\delta^{18}O_{\text{leaf water}}$ by	
763	subtracting the using biosynthetic fractionation factors, and the distance of	
764	reconstructed leaf water to the GMWL defines tThe deuterium excess (d = $\delta^2 H$ -	
765	8. $\delta^{18}O_$	Formatiert: Tiefgestellt
766	precipitation for each site are is calculated as intersection of the individual evaporation	
767	lines (ELs <sub>4</sub> (slope 2.82) with the global meteoric water line (GMWL).	
768	Fig. 6: Comparison of reconstructed humiditybiomarker-based on 'reconstructed' relative	
769	humidity (RH) values a normalized Craig Gordon model accounting for deuterium	
770	excess and temperature with modern-actual humidity dataRH values (mean annual RH	
771	retrieved for allthe investigated sites (from GeoINTA, 2012; summer daytime RH for	
772	six stations retrieved from www.ncds.noaa.gov). Deuterium excess values were	
773	calculated using $\delta^{18}O_{\text{leaf water}}$ reconstructed from terrestrial sugars (Tuthorn et al., 2014)	
774	and $\delta^2 H_{\text{leaf water}}$ reconstructed from <i>n</i> -alkanes.	
775	Fig. 7: Correlation of <u>biomarker-based</u> 'reconstructed' $\delta^{18}O_{prec}$ and $\delta^{2}H_{prec}$ values	Formatiert: Schriftart: Nicht Fett
776	reconstructed from the biomericans with entrol modern (entrol) (180 2)	Formatiert: Schriftart: Nicht Fett
//0	reconstructed from the promarkers with actual modern <u>actual</u> $\partial^{10}$ Uprec and $\partial^{2}$ Hprec	

777 <u>values (from Bowen, 2012), a and b, respectively.</u>