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Revisiting Mt. Kilimanjaro: Do *n*-alkane biomarkers in soils reflect the $\delta^2\text{H}$ isotopic composition of precipitation?

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7823

Abstract

During the last decade compound-specific deuterium (^2H) analysis of plant leaf wax-derived *n*-alkanes has become a promising and popular tool in paleoclimate research. This is based on the widely accepted assumption that *n*-alkanes in soils and sediments generally reflect $\delta^2\text{H}$ of precipitation ($\delta^2\text{H}_{\text{prec}}$). Recently, several authors suggested that $\delta^2\text{H}$ of *n*-alkanes ($\delta^2\text{H}_{n\text{-alkanes}}$) can also be used as proxy in paleoaltimetry studies.

Here we present results from a $\delta^2\text{H}$ transect study (~ 1500 to 4000 m a.s.l.) carried out on precipitation and soil samples taken from the humid southern slopes of Mt. Kilimanjaro. Contrary to earlier suggestions, a distinct altitude effect in $\delta^2\text{H}_{\text{prec}}$ is present above ~ 2000 m a.s.l., i.e. $\delta^2\text{H}_{\text{prec}}$ values become more negative with increasing altitude. The compound-specific $\delta^2\text{H}$ values of $n\text{C}_{27}$ and $n\text{C}_{29}$ do not confirm this altitudinal trend, but rather become more positive both in the O-layers (organic layers) and the A_h -horizons (mineral topsoils). Although our $\delta^2\text{H}_{n\text{-alkane}}$ results are in agreement with previously published results from the southern slopes of Mt. Kilimanjaro (Peterse et al., 2009, BG, 6, 2799–2807), a major re-interpretation is required given that the $\delta^2\text{H}_{n\text{-alkane}}$ results do not reflect the $\delta^2\text{H}_{\text{prec}}$ results. The theoretical framework for this re-interpretation is based on the evaporative isotopic enrichment of leaf water associated with transpiration process. Modelling results show that relative humidity, decreasing considerably along the southern slopes of Mt. Kilimanjaro (from 78 % at ~ 2000 m a.s.l. to 51 % at 4000 m a.s.l.), strongly controls $\delta^2\text{H}_{\text{leafwater}}$. The modelled ^2H leaf water enrichment along the altitudinal transect matches well the measured ^2H leaf water enrichment as assessed by using the $\delta^2\text{H}_{\text{prec}}$ and $\delta^2\text{H}_{n\text{-alkane}}$ results and biosynthetic fractionation during *n*-alkane biosynthesis in leaves.

Given that our results clearly demonstrate that *n*-alkanes in soils do not simply reflect $\delta^2\text{H}_{\text{prec}}$ but rather $\delta^2\text{H}_{\text{leafwater}}$, we conclude that care has to be taken not to over-interpret $\delta^2\text{H}_{n\text{-alkane}}$ records from soils and sediments when reconstructing $\delta^2\text{H}$ of

7824

paleoprecipitation. Both in paleoaltimetry and in paleoclimate studies changes in relative humidity and consequently in $\delta^2\text{H}_{n\text{-alkane}}$ values can completely mask altitudinally or climatically-controlled changes in $\delta^2\text{H}_{\text{prec}}$.

1 Introduction

5 It is well-known that the isotopic composition of precipitation ($\delta^2\text{H}_{\text{prec}}$ and $\delta^{18}\text{O}_{\text{prec}}$) has strong ties to climatic parameters such as temperature, amount of precipitation, relative humidity of the atmosphere, and some others (e.g. Araguas-Araguas et al., 2000; Craig, 1961; Dansgaard, 1964; Gat, 1996; Rozanski et al., 1993). The global and regional variability of the isotopic composition of precipitation is usually described
10 in terms of empirical “effects” illustrating the dependence of $\delta^2\text{H}_{\text{prec}}$ and $\delta^{18}\text{O}_{\text{prec}}$ on climatic and environmental factors. The most important ones are the latitude effect, the continental effect, the amount effect, the temperature effect and the altitude effect. Hence it is not surprising that both deuterium (^2H) and oxygen-18 (^{18}O) are used as paleoclimate and -altimetry proxies in different kinds of archives, such as ice cores,
15 speleothems, lake sediments and tree rings, in order to reconstruct climate history (Barker et al., 2011; Danis et al., 2006; Mayer and Schwark, 1999; Spötl et al., 2006; Thompson et al., 2002) and paleoaltitude (Mulch and Chamberlain, 2007; Rowley and Garzzone, 2007).

The application of these proxies to bulk plant, soil or sediment samples (Balabane,
20 1983; Ruppenthal et al., 2010), is, however, limited or at least complicated, because bulk material is composed of many different organic and inorganic pools characterised by different isotope values, resulting in a mixed isotope signal. Furthermore, certain pools are prone to post-sedimentary hydrogen exchange reactions (Schimmelmann et al., 2006; Zech, M. et al., 2011b). Therefore, when compound-specific $\delta^2\text{H}$ analyses
25 of organic marker molecules, so-called biomarkers became easier and faster thanks to the on-line coupling of gas chromatographs via a pyrolysis oven to isotope ratio mass spectrometers (GC-Py-IRMS) (Burgoyne and Hayes, 1998; Hilkert et al., 1999),

7825

the renewed interest in those proxies resulted in numerous new applications. A similar potential is offered by compound-specific $\delta^{18}\text{O}$ analyses (Zech, M. et al., 2012).

Since Sternberg (1988) reported that plant lipids record $\delta^2\text{H}$ of environmental water, the potential of sedimentary lipid biomarkers and their isotopic signature is evaluated
5 for paleoclimate studies (Huang et al., 2004; Sachse et al., 2004, 2012; Sauer et al., 2001; Sessions et al., 1999). For instance, *n*-alkanes which originate from terrestrial or aquatic plants (Eglinton and Hamilton, 1967; Ficken et al., 2000; Kolattukudy, 1976) are not prone to hydrogen exchange reactions in geologically young, thermally immature sediments (Dawson et al., 2007; Pedentchouk et al., 2006; Radke et al., 2005;
10 Sessions et al., 2004).

Recently, several studies investigating $\delta^2\text{H}$ of soil *n*-alkanes along altitudinal climate transects suggested that *n*-alkanes reflect $\delta^2\text{H}_{\text{prec}}$ and concluded that $\delta^2\text{H}$ of soil/sedimentary *n*-alkanes can be used for reconstructing paleoaltitudes as realized by Polissar et al. (2009), and more generally, for inferring $\delta^2\text{H}$ of paleoprecipitation
15 (Bai et al., 2011; Jia et al., 2008; Luo et al., 2011). However, there are also recent studies which identified various potential problems with the interpretation of $\delta^2\text{H}$ values of sedimentary *n*-alkanes. For instance, large plant-interspecies $\delta^2\text{H}$ differences under the same climatic conditions were observed (Feakins and Sessions, 2010; Hou et al., 2007; Liu et al., 2006; Smith and Freeman, 2006), as well as pronounced $\delta^2\text{H}$ variability
20 between different *n*-alkane homologues within one plant species. Also, seasonality of $\delta^2\text{H}$ of leaf wax *n*-alkanes was detected (Gao et al., 2012; Pedentchouk et al., 2008; Sachse et al., 2009). Finally, distinct differences between $\delta^2\text{H}$ values of soil and sediment *n*-alkanes compared to *n*-alkanes of respective fresh leaves (Chikaraishi and Naraoka, 2006; Sachse et al., 2006) and degradation effects on $\delta^2\text{H}$ of leaf litter by
25 soil microbial contributions to the long-chain *n*-alkane pool (Nguyen Tu et al., 2011; Zech, M. et al., 2011b) were observed.

The central aim of this work was to address the ambiguities indicated above in a focussed process study, thus contributing to the question: to which extent do *n*-alkane biomarkers in soils reflect the $\delta^2\text{H}$ isotopic composition of precipitation? This was done

by investigating $\delta^2\text{H}$ of precipitation and *n*-alkanes in soils along an altitudinal climate transect on the southern slopes of Mt. Kilimanjaro, East Africa. Although compound-specific $\delta^2\text{H}$ values of *n*-alkanes along this transect have already been published by Peterse et al. (2009), our data and associated modelling strongly suggest that a major re-interpretation of Peterse et al. (2009) data is required, taking into account previously neglected changes of climatically-controlled ^2H enrichment of leaf water in plants with altitude. The findings of this work have also wider implications for paleoclimate and paleoaltimetry studies based on $\delta^2\text{H}$ values of *n*-alkanes.

2 Material and methods

2.1 Study area

A detailed description of the study area was given previously by Hemp (2009), Zech, M. (2006) and Zech, M. et al. (2011a). Briefly, Mt. Kilimanjaro is an ancient volcano comprising three peaks (Shira, Mawenzi and Kibo) originating from three major former eruption periods. It is located about 300 km south of the equator in East Africa between $2^\circ 45'$ and $3^\circ 25'$ S and $37^\circ 0'$ and $37^\circ 43'$ E and rises from the surrounding savanna at about 700 m a.s.l. to the ice-covered summit with 5895 m a.s.l. The climate is bimodal characterised by two distinct rainy seasons in April/May and November and furthermore strongly depends on altitude and exposure. Mean annual temperature decreases with a nearly constant lapse rate from the foothills (23.4° , Moshi town) to the summit (-7.1°C). Mean annual precipitation (MAP) reaches a maximum (about 3000 mm yr^{-1}) on the southern slopes around 2200 m a.s.l. and declines rapidly both further uphill and downhill (Hemp, 2006). The soils in the montane forest belt have been classified as Andosols and are characterised by large stocks of soil organic carbon and the widespread occurrence of paleosol-sequences (Zech, M., 2006).

Climatic factors and topography cause a pronounced vegetational zonation, which was intensively studied by Hemp (2009, and literature therein). Very roughly, it com-

7827

prises the savanna (with mainly Ferralsols and Acrisols) and the intensively cultivated zone (mainly Anthrosols) below about 1900 m a.s.l., several distinct montane forest zones (up to about 3200 m a.s.l.), the subalpine zone (about 3200 m to 4000 m a.s.l.), which is characterised by its richness in *Erica* species, and finally the alpine zone (above about 4000 m a.s.l.).

During fieldwork in August 2005, an altitudinal soil transect ranging from 1430 to 4000 m a.s.l. was established along the southern slopes of Mt. Kilimanjaro (Table S1 and Fig. 1). Mixed samples composed of 5 subsamples from 19 study sites were taken for the organic layers (O-layers, not available for the lowermost and the uppermost study sites) and the humic-rich topsoils (A_h -horizons, sampled between 0 and 10 cm depth). While the two lowermost study sites are located in the cultivated zone (and the strongly disturbed forest 1430 and 1750 m a.s.l., respectively), the study sites from 2030 to 3060 m a.s.l. are located in the relatively undisturbed montane rainforest zone. Furthermore, two study sites were established at the transition to the subalpine zone (3150 and 3230 m a.s.l.) covered by *Erica excelsa* and *Hagenia abyssinica*, and two in a subalpine *Erica trimera* forest (3800 and 4000 m a.s.l.).

In order to quantify the altitude effect in the isotopic composition of precipitation along the southern slopes of Mt. Kilimanjaro, precipitation samples from 14 rain gauges located between 1430 and 3700 m a.s.l. were collected. The custom-build rain gauges were composed of a 20 L plastic canisters buried in the soil and covered with twigs and leaves to protect them from sunlight. Precipitation was collected using funnels installed at ~ 1.5 m above the local ground and passed to the canisters using flexible hoses which were sealed to the canisters using silicon. This design allowed unattended collection of cumulative samples representing several months of rainfall, without discernible isotope effects (see Sect. 3.1.). The samples were collected during 2008 and 2009 rainy periods.

2.2 Sample preparation and isotope analyses

2.2.1 Bulk isotope analyses

The rainwater samples were stored and transported in small, tightly closed plastic bottles, which were filled to the top. Water isotope analyses ($\delta^2\text{H}$ and $\delta^{18}\text{O}$) were carried out in the Laboratory of Isotope Biogeochemistry of the Bayreuth Center of Ecology and Environmental Research (BayCEER, University of Bayreuth, Germany) using a liquid autosampler and a TC-IRMS coupling (via a ConFlo IV interface, Thermo Fisher Scientific, Bremen, Germany) of a HTO pyrolysis oven (HEKAtech, Wegberg, Germany) with a Delta V Advantage IRMS (Thermo Fisher Scientific). Bulk $\delta^2\text{H}$ values of the soil samples were determined in the same laboratory after drying in a heating oven at 105 °C, thorough homogenization and grinding of the samples. For the thermal conversion, a TC/EA pyrolysis oven (HEKAtech) was coupled via a ConFlo III Interface (Thermo Fisher Scientific) with a Delta V Plus IRMS (Thermo Fisher Scientific). The standard deviation of bulk $\delta^2\text{H}$ analyses was typically less than $\pm 2\text{‰}$.

All $\delta^2\text{H}$ values are expressed as per mil (‰) deviations relative to the Vienna Standard Mean Ocean Water (VSMOW), based on an in-house reference gas (H_2 from Rießner-Gase GmbH, 96215 Lichtenfels, Germany, purity 6.0), which was calibrated using internationally accepted standards obtained from IAEA (VSMOW2, SLAP2 and IAEA-CH-7).

2.2.2 Compound-specific $\delta^2\text{H}$ analyses of *n*-alkanes

The *n*-alkanes were extracted from the soil samples in the Laboratory of the Department of Soil Physics, University of Bayreuth, Germany, according to a slightly modified procedure described by Zech, M. and Glaser (2008). Briefly, the procedure involves extraction of lipids with methanol/toluene (7/3) using an accelerated solvent extractor (ASE 200, Dionex, Germering, Germany) and purification of *n*-alkanes on

7829

silica/aluminium oxide (both 5 % deactivated) columns with hexane/toluene (85/15) as eluent.

$\delta^2\text{H}$ values of $n\text{C}_{27}$, $n\text{C}_{29}$ alkanes ($n\text{C}_{31}$ was less abundant and therefore did not withstand linearity criteria in many samples) recovered from the soil samples were determined in the Max Planck Institute, Jena, Germany, using a HP5890 gas chromatograph equipped with a DB-5 ms column (30 m $\times$ 0.32 mm i.d., 0.5 μm film thickness, Agilent) (Sachse et al., 2006). The gas chromatograph was coupled via a high-temperature conversion oven operated at 1425 °C (Hilkert et al., 1999) to an isotope ratio mass spectrometer (Delta^{plus}XL, Finnigan MAT, Bremen, Germany) for compound-specific $\delta^2\text{H}$ analyses. Each sample was analyzed in triplicate.

All $\delta^2\text{H}$ values were normalized to the VSMOW scale using a mixture of *n*-alkanes ($n\text{C}_{10}$ to $n\text{C}_{32}$). The $\delta^2\text{H}$ values of the *n*-alkanes in the standard mixture were calibrated against internal references (NBS-22, IAEA-OH22) using TC/EA-IRMS. The accuracy and drift of the system were evaluated via three standard measurements after every three samples (nine injections). To ensure stable ion source conditions the H_3^+ factor was determined at least once a day.

3 Results and discussion

3.1 $\delta^2\text{H}$ and $\delta^{18}\text{O}$ of precipitation: altitude effect on Mt. Kilimanjaro

The measured isotopic composition of precipitation at 14 stations along the southern slopes of Mt. Kilimanjaro is summarised in Table S2 and illustrated in a $\delta^2\text{H}$ – $\delta^{18}\text{O}$ diagram in Fig. 2. The $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values vary from -9.3 to -30.6‰ and from -2.9 to -5.5‰ , respectively. The deuterium(d-) excess values ($d = \delta^2\text{H} - 8 \times \delta^{18}\text{O}$) defining the position of the data points with respect to the Global Meteoric Water Line vary from $+12.0$ to $+17.1\text{‰}$ (Fig. 2). These values indicate that the collection and subsequent handling of precipitation samples proceeded without discernible isotope effects. The mean d-excess value is equal $+14.6\text{‰}$.

7830

Changes of $\delta^2\text{H}_{\text{prec}}$ with altitude are shown in Fig. 3b. Included are also the values representing snow samples collected few hours after the snowfall on 12 February 1969 (Tongiorgi, 1970). All those samples represent the same snow event. Only $\delta^{18}\text{O}$ was measured at that time. $\delta^2\text{H}_{\text{prec}}$ values shown in Fig. 3b were calculated from the available $\delta^{18}\text{O}$ data using the quoted above mean d-excess value equal 14.6‰. It is clear from Fig. 3b that, contrary to earlier suggestions (Peterse et al., 2009; Rietti-Shati et al., 2000) a distinct altitude effect is present on the southern slopes of Mt. Kilimanjaro above ca. 2000 m a.s.l.

Figure 3b contains also $\delta^2\text{H}_{\text{prec}}$ values representing Mt. Cameroon, an isolated ancient volcano in equatorial Africa (4°13' N, 9°10' E), rising to 4095 m a.s.l. in direct vicinity of the Atlantic coast (Gonfiantini et al., 2001). The $\delta^2\text{H}_{\text{prec}}$ values on Mt. Cameroon decrease with increasing altitude with the slope of -14.1‰ km^{-1} , to be compared with -14.9‰ km^{-1} observed for Mt. Kilimanjaro above ca. 2000 m a.s.l. For comparison, Jia et al. (2008) assumed a $\delta^2\text{H}$ lapse rate for precipitation of $\sim -16\text{‰ km}^{-1}$ at Mt. Gongga, China, and Araguas-Araguas et al. (2000) reported $\delta^2\text{H}$ lapse rates ranging from ~ -10 to 40‰ km^{-1} .

Gonfiantini et al. (2001) have shown that changes of $\delta^2\text{H}_{\text{prec}}$ and $\delta^{18}\text{O}_{\text{prec}}$ with altitude on Mt. Cameroon can be faithfully reproduced by a Rayleigh-type model with pseudo-adiabatic ascent of moist maritime air masses arriving from the Atlantic Ocean. The changes of $\delta^2\text{H}_{\text{prec}}$ and $\delta^{18}\text{O}_{\text{prec}}$ with altitude predicted by the model agree very well with the observed ones. Similar lapse rates of $\delta^2\text{H}_{\text{prec}}$ observed at both mountains suggest that $\delta^2\text{H}_{\text{prec}}$ values observed at Mt. Kilimanjaro above ca. 2000 m a.s.l. can be explained by the analogous physical mechanism, i.e. pseudo-adiabatic ascent of moist air masses along the southern slopes of the mountain toward the summit, associated with cloud formation and subsequent rain-out.

There are, however, important differences between the two $\delta^2\text{H}_{\text{prec}}$ curves shown in Fig. 3b. First, the lowermost part of the Mt. Kilimanjaro curve, up to ca. 2000 m a.s.l., does not reveal any altitude effect. On the contrary, the deuterium content in rainfall

7831

increases slightly with increasing altitude, despite the fact that amount of precipitation increases from 1800 mm at 1430 m to 2690 mm at 2130 m a.s.l. (Table S2). It is worth to note that rainfall recorded on Mt. Cameroon at 10 m a.s.l. reveals a more negative $\delta^2\text{H}$ value than the rainfall recorded at Mt. Kilimanjaro at ca. 2300 m a.s.l. Second, although Mt. Kilimanjaro is located ~ 350 km inland (to be compared with Mt. Cameroon located in the immediate vicinity of the source of moisture i.e. Atlantic Ocean), the $\delta^2\text{H}_{\text{prec}}$ values recorded at the given altitude on Mt. Kilimanjaro are significantly more enriched when compared to Mt. Cameroon. The difference between both $\delta^2\text{H}_{\text{prec}}$ curves increases with altitude, from ca. 10‰ at 1500 m to approximately 35‰ above 2300 m a.s.l.

These striking differences between those two $\delta^2\text{H}_{\text{prec}}$ curves presented in Fig. 3b can be reconciled if the role of recycled (transpired) moisture in the local atmospheric water balance is taken into account. It is well-known that the isotopic composition of transpired moisture is similar to that of the source water (see Sect. 3.4), thus being isotopically much heavier than the vapor of marine origin. Consequently, in regions where the local atmospheric water budget is dominated by moisture transpired by plant cover, the isotopic composition of atmospheric water vapor and the resulting rainfall will be isotopically much heavier than the rainfall produced from moisture of solely marine origin. This is apparently the case of the southern slopes of Mt. Kilimanjaro. Systematic observations and modelling performed by Pepin et al. (2010) clearly show that the montane rainforest belt extending on the southern slopes of Mt Kilimanjaro from approximately 1900 up to 3200 m a.s.l., is an important source of moisture for the regional atmosphere. This moisture is advected towards the summit, mixes with regional free-air water vapor and undergoes partial rain-out on the slopes of the mountain. It also mixes with the atmosphere beneath the forest belt. The importance of transpired moisture for the regional atmospheric water balance over eastern Africa has been pointed out by Rozanski et al. (1996) in the context of Addis Ababa (Ethiopia) rainfall, which is exceptionally enriched in heavy isotopes.

7832

Slightly growing $\delta^2\text{H}_{\text{prec}}$ values between ca. 1430 and 2000 m.a.s.l. can stem from partial rain-out of the atmospheric water vapor pool that is characterised by increasing proportion of transpired moisture with decreasing distance to rainforest belt. Decreasing condensation temperature (if occurs) can also contribute to the observed effect.

3.2 $\delta^2\text{H}$ of bulk soil material: “pool effects”

Bulk $\delta^2\text{H}$ values of the O-layers and the A_h -horizons shown in Fig. 4a reveal partly similar trends along the altitudinal transect, i.e. minima between 2500 and 3000 m.a.s.l., but they do not correlate significantly along the whole transect ($R^2 = 0.08$, $p = 0.272$). While $\delta^2\text{H}$ of the O-layers roughly reflects $\delta^2\text{H}_{\text{prec}}$, bulk $\delta^2\text{H}$ of the A_h -horizons does not. Rather, bulk $\delta^2\text{H}$ of the A_h -horizons is strongly ($R^2 = 0.84$, $p < 0.001$) and negatively correlated with the TOC/N (total organic carbon/nitrogen) ratio. Figure 4b depicts that albeit less significantly, also bulk $\delta^2\text{H}$ of the O-layers is correlated with the TOC/N ratio ($R^2 = 0.33$, $p = 0.016$).

These findings suggest that particularly bulk $\delta^2\text{H}$ of mineral soils is strongly influenced by soil properties, and not only by $\delta^2\text{H}_{\text{prec}}$. Two hydrogen pool effects have to be addressed. First, hydrogen atoms exist in soils in exchangeable and non-exchangeable pools (Schimmelmann et al., 2006). Given the possible transfer of hydrogen from soil water to organic and inorganic hydrogen soil pools and vice versa, bulk $\delta^2\text{H}$ values of soils can per se not archive original $\delta^2\text{H}$ values of plant-derived organic matter. An interesting approach to overcome this problem may therefore be the sample equilibration method proposed by Schimmelmann (1991) and Ruppenthal et al. (2010), which allows determining $\delta^2\text{H}$ values for the non-exchangeable hydrogen soil pools only. Second, distinct organic pools have different $\delta^2\text{H}$ values, e.g. lipids are relatively ^2H -depleted, whereas carbohydrates are relatively ^2H -enriched. Hence, even non-exchangeable soil hydrogen may be prone to pool size effects when the litter input varies qualitatively or when soil organic matter degradation affects distinct soil hydrogen pools differently.

7833

3.3 $\delta^2\text{H}$ of *n*-alkane biomarkers

The $\delta^2\text{H}$ values of *n*-alkane biomarkers obtained in the framework of this study are summarised in Table S1. The mean $\delta^2\text{H}$ values and the standard deviations of triplicate measurements are reported. While the peaks in the chromatograms for $n\text{C}_{31}$ were often too small to be evaluated reliably, compound-specific $\delta^2\text{H}$ results for $n\text{C}_{27}$ and $n\text{C}_{29}$ were obtained for all samples and range from -162 to -102‰ and from -163 to -107‰ in the O-layers and A_h -horizons, respectively (Fig. 5). However, when data points, which presumably do not withstand the linearity criteria (peak amplitudes smaller than 750 mV) are excluded, the $\delta^2\text{H}$ range becomes much more narrow. We assume that those data points reflect methodological artefacts, which are caused by low *n*-alkane (and TOC) concentrations in the respective soil samples and hence low *n*-alkane peaks in the chromatograms. Further studies are needed and encouraged to check more carefully the sample-size effect of the $\delta^2\text{H}$ measurements. Possibly, a sample-size correction of the type proposed by Zech, M. and Glaser (2008) for compound-specific $\delta^{13}\text{C}$ analyses of *n*-alkanes could be helpful.

As seen in Fig. 5, the $\delta^2\text{H}$ values show similar trends for the O-layers and the A_h -horizons both for $n\text{C}_{27}$ ($R^2 = 0.22$, $p = 0.064$) and $n\text{C}_{29}$ ($R^2 = 0.18$, $p = 0.112$), respectively. While the $\delta^2\text{H}$ values of $n\text{C}_{29}$ reveal no systematic trend with altitude, the $\delta^2\text{H}$ values of $n\text{C}_{27}$ become slightly more positive with altitude, thus corroborating the results of Peterse et al. (2009). Their $\delta^2\text{H}_{\text{leafwax}}$ data (weighted mean of $n\text{C}_{29}$, $n\text{C}_{31}$ and $n\text{C}_{33}$) are also shown in Fig. 5 and lacking measured $\delta^2\text{H}_{\text{prec}}$ values were interpreted to reflect the isotopic composition of precipitation. However, our results clearly show that *n*-alkanes in soils do not reflect $\delta^2\text{H}_{\text{prec}}$ values along the southern slope of Mt. Kilimanjaro. A reinterpretation is hence required and proposed in the next section.

7834

3.4 Model-data comparison: *n*-alkanes in soils reflect $\delta^2\text{H}_{\text{leaf water}}$

Like the ^{18}O isotopic composition of (hemi-)cellulose (Tuthorn et al., 2014; Zech, M. et al., 2013), the deuterium isotopic composition of plant biomarkers can be expected to depend on three main factors: (i) the ^2H content in source water, (ii) the ^2H evaporative enrichment of leaf water due to isotope effects associated with phase change and transport of water during transpiration, and (iii) a ^2H isotope fractionation associated with biosynthesis of plant biomarkers. It is generally accepted that uptake of soil water by plants is not associated with discernible isotopic fractionation (Dawson et al., 2002; Wershaw et al., 1966). Hence, in cases where significant evaporative enrichment of soil water can be excluded, the deuterium isotopic composition of the source water should reflect the mean isotopic composition of local precipitation ($\delta^2\text{H}_{\text{source water}} \cong \delta\text{H}_{\text{prec}}$).

Given that *n*-alkane biomarkers are synthesised in leaves, they reflect the isotopic composition of leaf water (Kahmen et al., 2013), albeit with a systematic offsets of approximately -160‰ (Sachse et al., 2006; Sessions et al., 1999) referred to as biosynthetic fractionation. In the course of the transpiration process, leaf water is becoming progressively enriched in ^2H , quickly reaching isotopic steady-state (e.g. Flanagan et al., 1991; Farquhar et al., 2007) which is a function of relative humidity, isotopic composition of the source (soil) water and isotopic composition of local atmospheric water vapor. Although over the years different models have been proposed to describe the isotopic enrichment of leaf water (e.g. Flanagan et al., 1991; Kahmen et al., 2011; Roden et al., 2000), they all rely on the conceptual framework for the isotope effects accompanying evaporation processes, laid down by Craig and Gordon (Craig and Gordon, 1965; Horita et al., 2008) and require an array of input parameters characterizing the evaporation process under given environmental conditions.

To calculate the expected ^2H isotope composition of leaf water we use here an analogy of transpiring leaf to a terminal lake i.e. the lake which is losing water only via evaporation. It can be shown that, under hydrologic and isotope steady-state conditions, the following approximate expression describes the ^2H isotope enrichment of

7835

a terminal lake (leaf water) (e.g. Gat and Bowser, 1991; Zech, M. et al., 2013):

$$\Delta\delta^2\text{H} = \delta^2\text{H}_{\text{leaf water}} - \delta^2\text{H}_{\text{source water}} \cong (1 - h_N)\varepsilon_2^* + \Delta^2\varepsilon \quad (1)$$

where:

- h_N – relative humidity of the atmosphere, normalized to the temperature of the leaf
- $\delta^2\text{H}_{\text{leaf water}}$ – steady-state isotopic composition of the leaf water (‰)
- $\delta^2\text{H}_{\text{source water}}$ – isotopic composition of the source water equal to mean isotopic composition of precipitation (‰)
- ε_2^* – equilibrium ^2H enrichment $(1 - 1/\alpha_{L/V}) \cdot 10^3$. $\alpha_{L/V}$ stands for equilibrium ^2H isotope fractionation between liquid and gaseous phase (‰)
- $\Delta^2\varepsilon$ – kinetic isotope enrichment ($\Delta^2\varepsilon = C_k^2(1 - h_N)$) where C_k^2 stands for kinetic enrichment parameter.

It is worth to note here that, in the framework of terminal lake analogy, the isotope steady-state assumption implies that isotopic composition of the transpired water vapor is equal to the isotopic composition of the source water.

Equation 1 was used to calculate the isotope enrichment of leaf water ($\Delta\delta^2\text{H} = \delta^2\text{H}_{\text{leaf water}} - \delta^2\text{H}_{\text{prec}}$) along an altitudinal transect on the southern slopes of Mt Kilimanjaro. The $\Delta\delta^2\text{H}$ values were calculated for the altitudes at which the soil samples were collected. $\delta^2\text{H}_{\text{prec}}$ values at those altitudes were obtained by interpolation of the data available for rain gauge stations (Table S2). Ground-level air temperatures at those altitudes were obtained by interpolating the mean temperatures for the period 2004–2012, representing nine stations situated on south-western slopes of Mt. Kilimanjaro and covering the altitude range from 1890 to 5470 m a.s.l. (N. Pepin – unpublished data; see also Pepin et al., 2010). The normalized relative humidities (h_N) as a function of altitude were calculated using the above indicated ground-level temperatures and free-air water vapor pressures at the altitudes corresponding to the sites where soil samples were

7836

collected, derived from the data reported by Pepin et al. (2010). The leaf temperatures were assumed to be equal ground-level air temperatures. Equilibrium isotope enrichments ε_2^* as function of temperature were calculated using empirical equation of Horita and Wesolowski (1994) describing temperature dependence of equilibrium fractionation factor $\alpha_{L/V}$ for deuterium. Since transport of water vapor from the leaf interior to the atmosphere and from the atmosphere to the leaf occurs via narrow stomata openings favoring dully-developed diffusive layer, it has been assumed in the calculations that C_k^2 is equal to its maximum value (25.1 ‰ – Merlivat, 1978).

The modelling results are summarised in Table S3. Reported are $\Delta\delta^2\text{H}$ values as a function of altitude, calculated using Eq. (1). Table S3 contains also the measured $\Delta\delta^2\text{H}$ values derived using weighted means of $\delta^2\text{H}$ of $n\text{C}_{27}$ and $n\text{C}_{29}$ alkanes representing A_h -horizons in the soil samples collected in the framework of this study, corrected for biosynthetic fractionation equal -160 ‰. The source water $\delta^2\text{H}$ values were assumed to be equal local $\delta^2\text{H}_{\text{prec}}$ values, derived by interpolation of $\delta^2\text{H}_{\text{prec}}$ data available for rain gauge stations.

The modelled and measured $\Delta\delta^2\text{H}$ values are plotted in Fig. 6 as a function of altitude. It is clear from Fig. 6 that the calculated values of leaf water ^2H enrichment as a function of altitude match the measured values relatively well despite a simplified representation of the ^2H isotope effects and transformations occurring along the route from precipitation through soil water and leaf water, to the isotopic imprint in n -alkanes, embedded in Eq. (1). Although for some altitudes the modelled $\Delta\delta^2\text{H}$ values differ significantly from the measured ones, the general trend is captured very well. These differences most probably stem from inadequate representation of relative humidity at those sites. It should be noted that $\Delta\delta^2\text{H}$ defined by Eq. (1) is a sensitive function of relative humidity; it changes by ca. 1.1 ‰ by 1 % change in this parameter. Therefore, it would be relatively easy to get a perfect match of measured and modelled $\Delta\delta^2\text{H}$ values by manipulating the relative humidity. The required changes would be within 10 % range with respect to the altitudinal profile of h_N adopted in the calculations (c.f. Table S3). Since h_N in Eq. (1) signifies the relative humidity of the local atmosphere

7837

seen by leave water and normalized to its temperature, it is virtually impossible to get exact, representative values from direct measurements. It is also clear from Fig. 6 that increasing evaporative enrichment of the source water (precipitation) in leaves, driven by decreasing relative humidity, can completely mask the isotope signal imprinted in precipitation, created by the local altitude effect.

Figure 6 contains also the modelled altitudinal profile of $\Delta\delta^2\text{H}$ for the hypothetical case of constant relative humidity (RH = 75 %). A purely vertical line would mean perfect representation of $\delta^2\text{H}_{\text{prec}}$ changes with altitude by the $\delta^2\text{H}$ values of n -alkanes. The slight increase of $\Delta\delta^2\text{H}$ with increasing altitude seen in Fig. 6 (ca. 3 ‰ between 2000 and 4000 m) is due to the temperature dependence of the equilibrium fractionation factor for deuterium. The comparison of these two model scenarios (i) highlights the strong influence of changes in relative humidity on $\delta^2\text{H}_{\text{leaf water}}$ and thus also on $\delta^2\text{H}_{n\text{-alkanes}}$ and (ii) confirms the validity of the conceptual model used for our $\delta^2\text{H}_{n\text{-alkane}}$ data interpretation.

4 Conclusions

Revisiting the Mt. Kilimanjaro paid-off in the sense that the results of the presented process study shed new light on the interpretation of compound-specific $\delta^2\text{H}_{n\text{-alkane}}$ data obtained for soils along steep ecological and climatic gradients. Lacking measured $\delta^2\text{H}_{\text{prec}}$ values, Peterse et al. (2009), on the basis of their $\delta^2\text{H}_{\text{leaf wax}}$ data and earlier suggestions of Rietti-Shati et al. (2000), claimed that (i) the altitude effect is absent on the southern slopes of Mt. Kilimanjaro and (ii) that n -alkanes reflect $\delta^2\text{H}_{\text{prec}}$. The above discussion clearly shows that this does not hold anymore.

- A distinct altitude effect in $\delta^2\text{H}_{\text{prec}}$ is present on the southern slopes of Mt Kilimanjaro above ca. 2000 m a.s.l. This effect, characterised by $\delta^2\text{H}_{\text{prec}}$ altitude gradient in the order of -15 ‰ km^{-1} , is consistent with findings from another tropical mountain (Mt. Cameroon) located in the immediate vicinity of the Atlantic Ocean.

7838

As shown by Gonfiantini et al. (2001) the gradient observed on Mt. Cameroon can be faithfully reproduced in the framework of Rayleigh-type modelling of ascending moist air masses, leading to formation of clouds and partial rain-out. The same can be applied to Mt. Kilimanjaro. The Mt. Kilimanjaro $\delta^2\text{H}_{\text{prec}}$ data revealed, however, two important differences with respect to Mt. Cameroon data (positive offset in the order of 35 ‰ above ca. 2000 m a.s.l. and reversed altitude effect below this altitude), both linked to a significant contribution of recycled (transpired) moisture in the local atmospheric water balance of Mt. Kilimanjaro.

- The theoretical framework for the interpretation of $\delta^2\text{H}_{n\text{-alkane}}$ data presented in this study highlights the dominant role of relative humidity of the local atmosphere in controlling ^2H enrichment of leaf water and, consequently, the isotope signature of organic compounds being synthesized in leaves. The presented results clearly demonstrate that enhanced evaporative ^2H enrichment of leaf water along an altitudinal gradient, steered by decreasing relative humidity, can completely mask the reduction of deuterium content in the source water (precipitation) induced by the altitude effect. So the central question of this study has to be answered as follows: n -alkanes in soils do not necessarily reflect $\delta^2\text{H}_{\text{prec}}$ but rather $\delta^2\text{H}_{\text{leaf water}}$. This finding is well in agreement with the interpretation of compound-specific $\delta^{18}\text{O}$ results of hemicellulose-derived sugar biomarkers extracted from topsoils along an Argentinian climate transect (Tuthorn et al., 2014).
- Furthermore, the findings of this study strongly suggest that great care is needed when compound-specific $\delta^2\text{H}$ data are used in paleoaltimetry and paleoclimate studies (practised exemplarily e.g. by Liu and Huang, 2005a and Zech, R. et al., 2013). In particular, it is clear from the presented data that independent a priori information is required with respect to possible changes of atmospheric relative humidity with altitude in the investigated area(s) in the past. Also climatically-driven changes of deuterium content in local precipitation as well as changes of temperature with altitude need to be taken into account. A way-out of this dilemma would

7839

be a coupled $\delta^2\text{H}$ - $\delta^{18}\text{O}$ approach in paleoaltimetry and paleoclimate studies as suggested by Zech, M. et al. (2013).

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7846

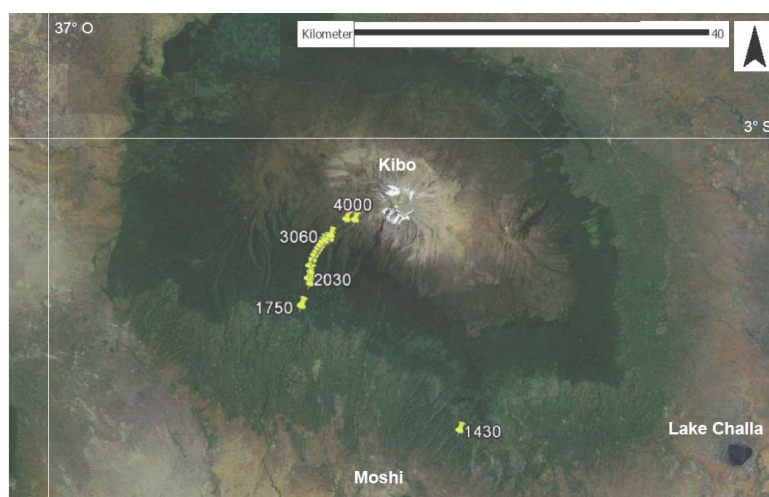


Figure 1. Satellite image of Mt. Kilimanjaro depicting the location of the 19 sites on the southern slopes of Mt. Kilimanjaro where soil and precipitation samples were collected (from GoogleEarth).

7847

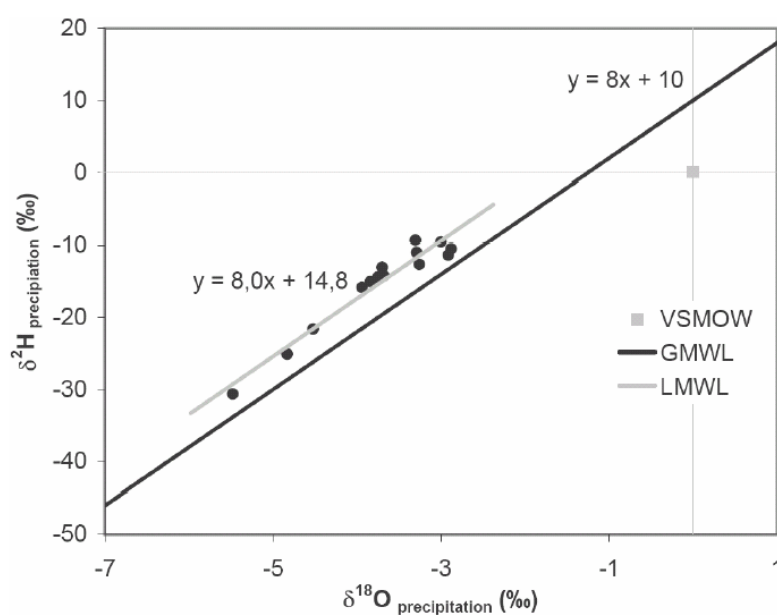


Figure 2. $\delta^2\text{H}$ - $\delta^{18}\text{O}$ relationship for precipitation samples collected on the southern slopes of Mt. Kilimanjaro during 2008–2009. GMWL = Global meteoric water line, LMWL = Local meteoric water line, VSMOW = Vienna standard mean ocean water.

7848

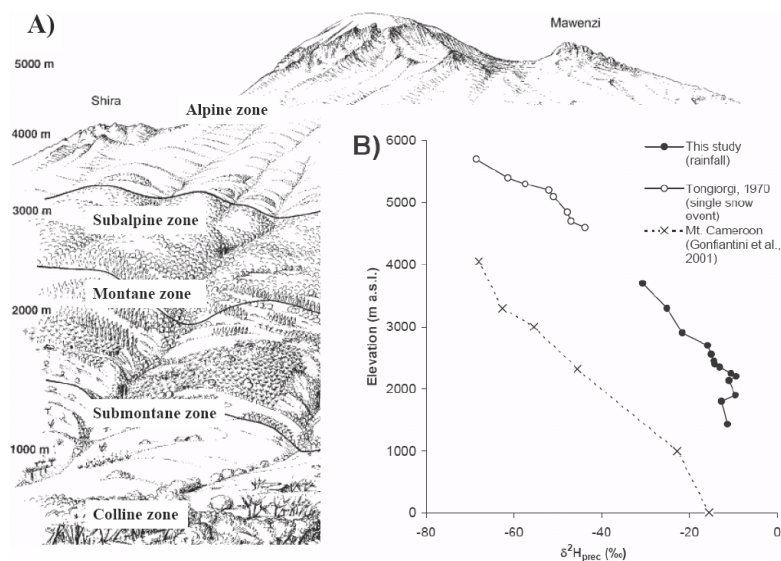


Figure 3. (A) Simplified altitudinal zonation of the vegetation characterizing the southern slopes of Mt. Kilimanjaro (from Hemp and Beck, 2001). (B) Change of $\delta^2\text{H}_{\text{prec}}$ with altitude on the southern slopes of Mt. Kilimanjaro and on the western slopes of Mt. Cameroon.

7849

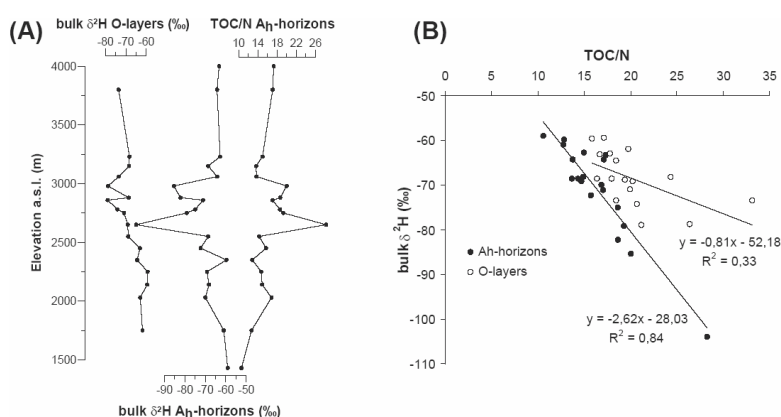


Figure 4. (A) Change of bulk $\delta^2\text{H}$ values of O-layers and A_h-horizons, respectively, and TOC/N (total organic carbon/nitrogen) ratios of A_h-horizons with altitude. (B) Correlation of bulk $\delta^2\text{H}$ values with TOC/N ratios.

7850

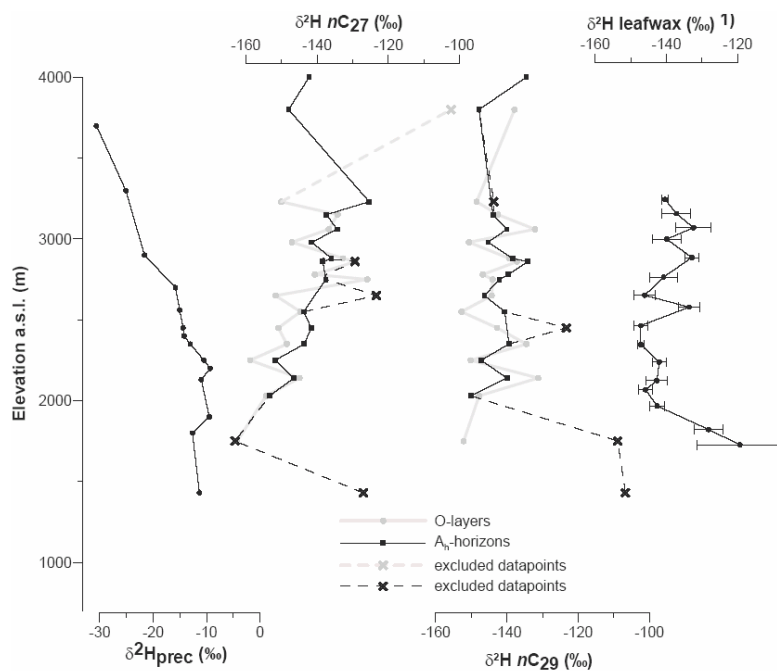


Figure 5. Change of $\delta^2\text{H}_{\text{prec}}$, $\delta^2\text{H}_{\text{nC27}}$ and $\delta^2\text{H}_{\text{nC29}}$ in the O-layers and the A_n -horizons, respectively, and $\delta^2\text{H}_{\text{leafwax}}$ with altitude. ¹⁾ From Peterse et al. (2009).

7851

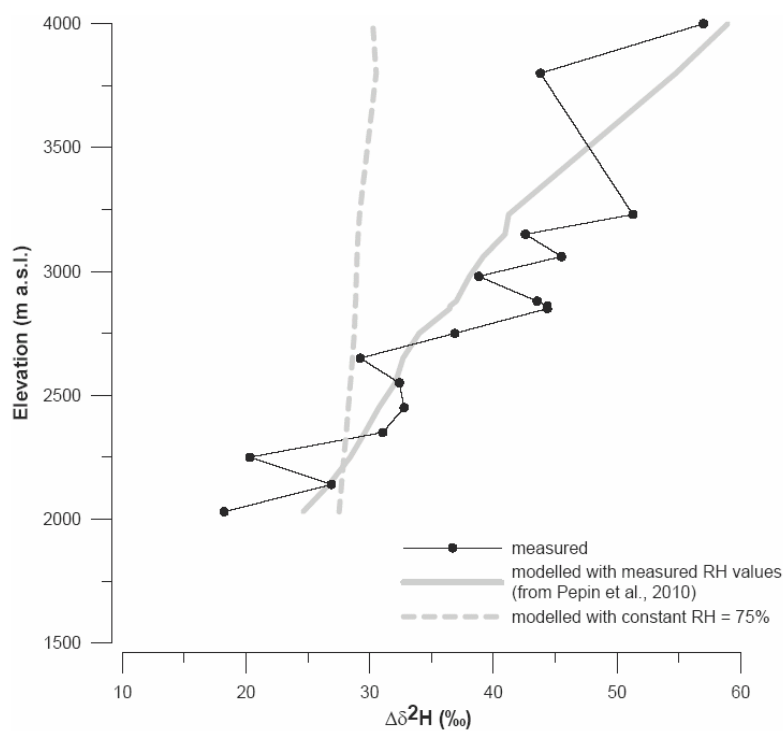


Figure 6. Comparison of measured and modelled $\Delta\delta^2\text{H}$ (^2H enrichment of leaf water) as a function of altitude along the southern slopes of Mt. Kilimanjaro. Modelled $\Delta\delta^2\text{H}$ values are derived from Eq. (1) (see text).

7852