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Plant water resource partitioning and xylem-leaf deuterium enrichment in a seasonally dry tropical climate

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Abstract. Lake Challa (3°19'S, 37°42'E) is a steep-sided crater lake situated in equatorial East Africa, a tropical semi-arid area with bimodal rainfall pattern. Plants in this region are exposed to a prolonged dry season and we investigated if 1) these plants show spatial variability and temporal shifts in their water source use; 2) seasonal differences in the isotopic composition of precipitation are reflected in xylem water; and 3) plant family, growth form, leaf phenology, habitat and season influence the xylem-to-leaf water deuterium enrichment. In this study, the δ^2H and $\delta^{18}O$ of precipitation, lake water, groundwater, plant xylem water and plant leaf water were measured across different plant species, seasons and plant habitats in the vicinity of Lake Challa. We found that plants rely mostly on water from the 'short' rains falling from October to December (northeastern monsoon), as these recharge the soil after the long dry season. This plant-available water pool is only slightly replenished by the 'long' rains falling from February to May (southeastern monsoon), in agreement with the 'two water world' hypothesis according to which plants rely on a static water pool while a mobile water pool recharges the groundwater. Trees at the lake shore and on the crater rim use more evaporated water than shrubs in the same habitats, suggesting that trees tap water from the topsoil where the nutrient content is highest. Plants at the lake shore rely on a water source admixed with lake water. The enrichment in deuterium from xylem water to leaf water averages 24 ± 28 ‰. According to our results, plant species and their associated leaf phenology are the primary factors influencing this enrichment factor, while growth form and season have negligible effects.

1 Introduction

35   The naturally occurring stable isotopes of hydrogen and oxygen in the water molecule have been highly
instructive as tracers in hydrology and eco-hydrology. This is mainly based on naturally occurring variations in
the relative abundance of two rare, heavy isotopes (i.e. ^{2}H or D and ^{18}O), arising from phase changes and mixing
as water passes through the hydrologic cycle. The hydrogen and oxygen isotopic composition of precipitation
varies both spatially and temporally, due to fluctuations i) at the site of evaporation, e.g. meteorological

40   conditions such as relative humidity (RH), wind and sea surface temperature; and ii) at the site of precipitation,
e.g. the degree of rainout of particular air masses (Craig, 1961; Dansgaard, 1964; Gat 1996; Araguas-Araguas et
al., 2000, Gibson et al., 2008). The stable isotopes of hydrogen and oxygen in precipitation show a distinct
empirical relationship, described by the Global Meteoric Water Line (GMWL: δ^2H = 8.1*δ^{18}O + 10.3 ‰;
Rozanski et al., 1993). The δ^{18}O-δ^2H relationship in precipitation at any single location is however better

45   described by a Local Meteoric Water Line (LMWL), which can have a different slope and intercept depending
on the conditions in which the local water source was formed. This is in turn used to compare different
(sub)surface water bodies with local precipitation (Rozanski et al., 1993; Breitenbach et al., 2010).

Variation of δ^2H or δ^{18}O in xylem water of plants has been used extensively to determine water sources used by
plants and their functional root depth. Water uptake through the roots and transport in plants is generally

50   considered to occur without fractionation (White et al., 1985) so that the isotopic composition of xylem water
represents the plant water source (Dawson & Ehleringer, 1991; Evaristo et al., 2015). Fractionation during root
water uptake has been found previously only for plants living under xeric conditions and in mangroves
(Ellsworth & Williams, 2007). The isotopic composition of leaf water differs markedly from that of xylem water
due to transpiration, as the isotopically lighter molecules in water vapor diffuse more readily than the heavier

55   ones. As a result, leaf water becomes more enriched in heavy isotopes. Ambient temperature, RH and the
isotopic composition of water vapor surrounding the plant mainly determine the enrichment from xylem to leaf
water (Kahmen et al., 2008; Sachse et al., 2012). Finally, evaporation directly from the soil changes the isotopic
composition of soil water available for plant roots.

The δD signature of *n*-alkanes, long-chain hydrocarbons with 25-35 carbon atoms, derived from fossil plant leaf

60   waxes incorporated in lake sediments is increasingly being used as paleoclimate proxy (Eglinton and Hamilton,
1967; Mayer and Schwark, 1999; von Grafenstein et al., 1999; Thompson et al., 2003; Tierney et al. 2008; Costa
et al. 2014). Consequently also a better understanding of the hydrogen fractionation is needed which occurs
during its incorporation from precipitation via leaf water into plant leaf waxes. The present study is developed in
the context of such an application of hydrogen isotope geochemistry for paleoclimate research, focusing on the

65   sediment record of Lake Challa in equatorial East Africa (Verschuren et al. 2009; Barker et al., 2011; Tierney et
al., 2011). In this semi-arid tropical region, biannual passage of the Intertropical Convergence Zone (ITCZ)
induces a strongly bimodal pattern of seasonal rainfall (Nicholson, 2000). Plants in this region are exposed to a
prolonged dry season between June and September during which little precipitation falls. Useful adaptations to
survive this period of water shortage include stem succulence, leathery leaves and deep roots providing access to

70   deep and permanent water sources (Elffers et al., 1964; Corbin et al., 2005). Meinzer et al. (1999) suggested that
competition for water may actually be limited (at least in pristine dryland ecosystems), due to the pronounced

spatial and temporal partitioning of water resources resulting from maximized species diversity. In any case it appears that the relationship between the root biomass in a particular soil layer and the degree of contribution of that soil layer to the plant's water budget is not always straightforward (Jackson et al., 1995; Stahl et al., 2013).

75   Therefore, dual stable isotope composition of xylem water could be valuable to elucidate the water sources effectively used by plants (Dawson & Ehleringer, 1991; Liu et al., 2010).

In this study, we measured the δ^2H and $\delta^{18}O$ of precipitation, lake water, groundwater, and of xylem and leaf water in several plants from three distinct habitats around Lake Challa, and this during successive wet and dry seasons of one complete year. Our primary research questions were: 1) are seasonal differences in the isotopic

80   composition of precipitation reflected in xylem water? 2) do plants show habitat-specific variability and temporal shifts in their water source use? and (3) what is the influence of plant family, growth form, phenology, season and habitat on the deuterium enrichment from xylem to leaf water?

2 Study site

Lake Challa is a 4.2 km², ~92 m deep crater lake with near-vertical inner crater walls (Moernaut et al., 2010),

85   situated on the southeastern slope of Mt. Kilimanjaro (3°19'S, 37°42'E) at 880 m above sea level (m.a.s.l.) in equatorial East Africa (Fig. 1). Biannual passage of the Intertropical Convergence Zone (ITCZ) induces a bimodal rainfall pattern with southeastern (SE) monsoon winds bringing 'long rains' normally from March to mid-May and northeastern (NE) monsoon winds bringing 'short rains' from late October to December (Nicholson, 2000; Fig. 2). The local climate is tropical semi-arid, with lowest mean monthly night- and daytime

90   temperatures in July-August (c. 18 and 28 °C, respectively) and highest in February-March (c. 21 and 33 °C, respectively; Fig. 1a) for Voi, 80 km east of Lake Challa (Buckles et al., 2014). Given the total annual rainfall of c. 565 mm and an estimated annual lake-surface evaporation of c. 1735 mm, the water budget of Lake Challa must be balanced by substantial groundwater input, which is mainly derived from precipitation falling onto montane forests of Mt. Kilimanjaro's east-facing slope at 1800-2800 m.a.s.l. (Payne, 1970; Hemp, 2006a).

95

The vegetation of the crater basin containing Lake Challa consists of different forest and woodland types (Hemp, 2006b). On the upper part of the inner slopes a dry forest occurs with succulents such as *Euphorbia quinquecostata* (Euphorbiaceae) and deciduous species such as *Commiphora baluensis* (Burseraceae) and *Haplocoelum foliolosum* (Sapindaceae) dominating the tree layer. Near the lake shore an evergreen forest with

100   *Sorindeia madagascariensis* (Anacardiaceae), *Ficus sycomorus* (Moraceae) and *Trichilia emetica* (Meliaceae) grows. In contrast, the outer crater slopes are covered with dry savanna woodlands, with a lower and more open canopy. The stunted, fruit tree-like appearance of the woody species -mainly belonging to Combretaceae, Burseraceae and Anacardiaceae- inspired the first botanists to describe this vegetation formation as 'Obstgartensteppe' ('fruittreegardensteppe'; Volkens, 1897). Whereas all these vegetation types grow on rocky

105   slopes with very shallow soils, the soils of the flat foothills are deeper. Here, most of the former natural savanna vegetation is converted into agricultural fields or meadows. The savanna woodlands still existing in this area are dominated by Acacias (*A. nilotica*, *A. senegal*; Mimosaceae).

3 Materials & methods

3.1 Sampling

110 Two duplicate rain gauges were installed in the savannah just outside Challa crater (at 3°19'S 37°42'E and 842 m.a.s.l.; Fig. 1) to sample precipitation monthly between September 2013 and August 2014. The collectors consisted of a 5 L plastic container with a plastic funnel of c. 15 cm diameter, in which a plastic fiber mesh net was placed to prevent dirt enter the bottle. A layer of mineral oil (thickness ca. 1.5 cm) was poured in the jars to avoid evaporation and exchange with air moisture, which might have altered the isotopic composition of the

115 collected water (Friedman et al., 1992). As only 6 precipitation samples reached the laboratory, precipitation was additionally collected in the savannah about 20 km to the west of the Challa crater (at 3°23'S 37°27'E and 820 m.a.s.l.) by an evaporation-free collector (Groning et al., 2012) of the brand PALMEX (Croatia) monthly from November 2014 until November 2015. Lake water was sampled 30 cm below the water surface in the middle of the lake, on a monthly basis from January 2013 until October 2014. A groundwater sample was obtained from

120 the Miwaleni spring (3°25'S 37°27'E) in July 2015.

Plant material was collected during the main dry season in September 2013 and July 2014, during the 'short' rain (NE monsoon) season in December 2013, during the short dry season in February 2014 and during the 'long' rain (SE monsoon) season in April 2014. However, because the 2014 long rain season atypically started already in February, our plant sampling for the short dry season was already influenced by fresh SE monsoon rainfall.

125 Fourteen plant species with varying growth form (grass, shrub or tree) and leaf phenology (deciduous or evergreen, the latter including succulent) were collected in three distinct habitats (savannah, crater rim, lake shore) around Lake Challa (Fig. 1, Table 1). Shrubs were defined as woody plants with multiple stems, while trees had one erect perennial stem. Lakeshore vegetation was sampled at the northeast side of the lake, savannah

130 was sampled outside the crater c. 500 m to the northwest, and crater-rim vegetation at the top of the crater's western rim (1100 m.a.s.l.). The locally most abundant plant species were chosen, although the choice of species was sometimes restricted by practical limitations such as difficulties in reaching certain locations. For each habitat, if possible at least three individuals of each species were sampled.

135 Two different sampling techniques were used to collect xylem water from plants, the choice of which depended on the plant type. When the plant had a trunk with a diameter of more than 10 cm, a core drill sample (300 mm, diameter 4.30 mm, hard-wood head, Pressler, Recklinghausen, Germany) was extracted, from which the outer layer (epidermis, cortex, bark fibres, and phloem) was removed. In the case of smaller trees and shrubs, a piece of twig was sampled from which the outer layer was scraped off using a knife before enclosing into sealed vials.

140 For leaf water analysis, leaves were taken from each plant and placed in vials. If twigs were sampled, leaves on those twigs were sampled. In case of core sampling, leaves were sampled at different heights and at different cardinal points, and one pooled sample was obtained. From the grasses only leaf water was obtained.

3.2 Analysis

145 Stem, twig, and leaf samples were stored frozen until the water was quantitatively extracted via cryogenic vacuum distillation (West et al., 2006). Following Araguàs-Araguàs et al. (1995), isotopic data was retained for

interpretation only if the extraction efficiency, determined by further drying of the sample at 105°C for at least 48 hours, exceeded 98%.

150   The δ²H and δ^{18}O values of water samples were determined using Cavity Ringdown Spectrometry (WS-CRDS, L2120-i, Picarro, USA). In this paper, isotopic composition is expressed in terms of ^{2}H/^{1}H and ^{18}O/^{16}O ratios, represented by δ values: $\delta_{sample} = (R_{sample}/R_{standard} - 1)$ with R_{sample} and $R_{standard}$ being the isotopic ratio (^{2}H/^{1}H or ^{18}O/^{16}O) measured in the sample and the standard, respectively (Gat, 2005). The used reference standard is Vienna Standard Mean Ocean Water (VSMOW) which has, by definition, a δ²H and δ^{18}O of 0 ‰. The

155   enrichment factor $\varepsilon_{l/x}$ characterizes the hydrogen-isotopic fractionation between xylem and leaf water and is defined as (Eq. 1):

$$\varepsilon_{l/x} = ((\delta^2 H_{leaf} + 1)/(\delta^2 H_{xylem} + 1)) - 1$$

160   Enrichment factors and δ values are typically reported in per mil (‰) (Cohen et al., 2007).

The average isotopic signature of the source of xylem water was determined from the intersection of xylem water samples (aligned along a local evaporation line, LEL) with the LMWL (Eq. 2 and 3):

$$\delta^{18}O_{LMWL-int} = \frac{\delta^2 H - slope_{LEL} * \delta^{18}O - intercept_{LMWL}}{slope_{LMWL} - slope_{LEL}}$$

$$\delta^2 H_{LMWL-int} = \delta^{18}O_{LMWL-int} * slope_{LMWL} + intercept_{LMWL}$$

165

The isotopic signatures of xylem were further characterized with a parameter describing the relative degree of evaporation, defined as ED and calculated as the distance from the LMWL along an evaporation line (Eq. 4). The higher this ED value, the more evaporated the water will be.

$$\text{Evaporation distance (ED)} = \sqrt{(\delta^2 H - \delta^2 H_{LMWL-int})^2 + slope_{LMWL} * \left(\delta^{18}O - \delta^{18}O_{LMWL-int}\right)^2}$$

170

Analyses of variance (ANOVA) were used for comparisons of δ^{2}H and δ^{18}O isotopic signatures among plant species, growth forms, leaf phenologies, seasons or habitats. Tukey post-hoc comparisons were used to further examine differences. All statistical analyses were performed using R (version 3.2.3.). Slopes and intercepts of LMWL and LEL were estimated with linear regressions. Discussion of these different slopes and intercepts are

175   not the scope of this paper, but were used to calculate δ^{2}H$_{LMWL-int}$.

4 Results

4.1 Isotopic composition of precipitation, lake water and groundwater

During our main sampling period from September 2013 until August 2014, local rainfall was highest in
180   December (peak 'short rains') and April (peak 'long rains') with 135 and 122 mm, respectively, and lowest in
January (short dry season) and August (long dry season, Fig. 2b). During the 12 month monitoring period, the
total amount of local precipitation was 692 mm. The monthly average temperature varied slightly between
23.9 °C in June-July and 27.1 °C in April, resulting in an overall annual mean of 25.5 ± 1.2 °C (mean $\pm 1\sigma$
standard deviation). The monthly minimum (night-time) temperature followed a similar pattern (19.6 ± 1.0 °C),
185   while the monthly maximum (day-time) temperature showed greater variability (37.5 ± 2.1 °C), with an atypical
minimum in February (34.8 °C) and maximum in April (41.2 °C).

The isotopic composition of precipitation (n = 6) varied by more than 84 ‰ for δ^2H and 11 ‰ for $\delta^{18}O$ during
the sample period (Fig. 2). Both δ^2H_{prec} and $\delta^{18}O_{prec}$ are most enriched during the dry month of July with values
190   of 36.6 and 4.2 ‰ and most depleted during rainy November with values of -47.9 and -7.2 ‰, respectively.
Comparing the two rainy seasons revealed considerable differences with more enriched rain during the SE
monsoon (δ^2H_{prec} of 16.0 ± 2.5 ‰ and $\delta^{18}O_{prec}$ of 0.8 ± 0.7 ‰; n = 3) compared to the NE monsoon (δ^2H_{prec} of -
26.5 ± 21.5 ‰ and $\delta^{18}O_{prec}$ of -4.9 ± 2.3 ‰; n = 2). In order to draw a reliable LMWL, precipitation samples (n =
12) covering the period November 2014 - November 2015 were added (from a savannah site 20 km west of Lake
195   Challa, cf. above). Based on this dataset (n = 12), the yearly volume-weighted average values are -6.5 ‰ for
δ^2H_{prec} and -2.5 ‰ for $\delta^{18}O_{prec}$. Compared to the global meteoric water line ($\delta^2H = 8.1*\delta^{18}O + 10.3$ ‰, Rozanski
et al., 1993), the local meteoric water line ($\delta^2H = 7.1*\delta^{18}O + 10.7$ ‰, n = 18) has a slightly lower slope and
higher intercept (Fig. 3).

200   The isotopic measurements on lake water during 22 consecutive months (from January 2013 until October 2014)
yielded mean δ^2H_{lake} and $\delta^{18}O_{lake}$ values of 17.4 ± 0.7 ‰ and 2.9 ± 0.2 ‰ respectively (Fig. 2 and 3), with very
little variation through the year but highest values in the warm months of February-March and a modest
minimum around August. The groundwater isotopic composition equaled -20.2 ‰ for δ^2H and -4.6 ‰ for $\delta^{18}O$
(Fig. 3).

205

4.2 Xylem water

The δ^2H of xylem water (δ^2H_{xylem}) in a total of 154 analyzed samples (no grasses) ranged between -87 and 25 ‰,
with an overall mean value of -18 ± 17 ‰ (Fig. 3). δ^2H_{xylem} varied considerably between habitats: δ^2H_{xylem} of
plants at the lake shore (-2 ± 10 ‰, n = 48) was isotopically more enriched compared to plants in the savannah (-
210   25 ± 12 ‰, p <0.01, n = 34) or on the crater rim (-26 ± 15 ‰, p <0.001, n = 72). Also the δ^2H_{xylem} of trees (3
species, n = 38) at the lake shore (1 ± 8 ‰) was significantly higher than that of the single shrub species sampled
in this habitat (-13 ± 5 ‰, p <0.001, n = 10). In the savannah and on the crater rim, no difference (p >0.05)
could be observed between the δ^2H_{xylem} of trees and shrubs. Across all sampled plants, leaf phenology (deciduous
or evergreen) caused no significantly different δ^2H_{xylem} value (p >0.05). Only two of the sampled species showed
215   an effect of season, but in a dissimilar pattern. The tree *Sideroxylon* sp. had lower δ^2H_{xylem} values during the long

dry season (0 ± 5 ‰, p <0.05) than during the short (10 ± 1 ‰) and long rain seasons (6 ± 8 ‰). The tree *Ficus sycomorus* showed lower values (p < 0.01) during the short rain season (-5 ± 2 ‰) than during the long dry season (4 ± 3 ‰) and long rain season (13 ± 4 ‰).

220 The hydrogen isotopic signatures of xylem samples follow an evaporation line (LEL). To determine the mean isotopic composition of the water source from which xylem water originated LELs were calculated for each of the three different plant habitats and used (Eq. 2 and 3) to estimate the intersection points of xylem water with the LMWL ($\delta^2H_{LMWL-int}$). LELs with a slope of about 3 fitted best with our data from savannah and the crater rim, while a slope of about 5 fitted best at the lake shore. The LELs with slope of 3 correspond well with the modeled

225 evaporation lines for soil water in our study area (Gibson et al. 2008). A slope of 5 corresponds more with these authors' modeled evaporation lines for surface water, which can be explained by the lake shore trees and shrubs mostly using lake water. The $\delta^2H_{LMWL-int}$ values ranged between -79 and -13 ‰, with an overall mean value of -41 ± 13 ‰. No statistical differences (p >0.05) could be observed between the $\delta^2H_{LMWL-int}$ values according to habitat, species, growth form or leaf phenology (Fig. S1). Plants at the lakeshore showed only a weak temporal

230 trend in $\delta^2H_{LMWL-int}$ (mean -45 ± 12 ‰, p >0.05), whereas plants in both the savannah (-42 ± 9 ‰, p <0.01) and on the crater rim (-38 ± 15 ‰, p <0.001) showed significant seasonal variability in $\delta^2H_{LMWL-int}$ (Fig. 4).

The evaporation distance (ED, Eq. 4) is a parameter describing the relative degree of evaporation, and is derived by calculating the distance of xylem data points from the LMWL along the LEL. A great range in ED values was

235 observed, varying between 1 and 94 ‰ across all samples (Fig. 5). Plants at the lakeshore produced systematically higher ED values (49 ± 13 ‰) than those in savannah (23 ± 14 ‰, p <0.001) or on the crater rim (17 ± 9 ‰, p <0.001). Also growth form influenced this parameter (p <0.05) with lower ED values for shrubs than for trees both at the lake shore (respectively 42 ± 16 ‰ and 50 ± 12 ‰) and on the crater rim (respectively 16 ± 9 ‰ and 22 ± 8 ‰, Fig. S2). No difference (p >0.05) in ED value was found between evergreen and

240 deciduous plants. The evaporation distance showed a clear temporal effect during the study period at the lake shore (p <0.01) and on the crater rim (p <0.01), however in the savannah the trend was not significant (p >0.05, Fig. 5). Finally, among the seven non-grass plant species sampled at the crater rim, a significant difference (p <0.01) was observed between the low ED value for *Vepris uguenensis* (9 ± 7 ‰) and the high ED value for *Euphorbia tirucalli* (25 ± 7 ‰).

245

4.3 Leaf water

The number of samples investigated for leaf water δ^2H (δ^2H_{leaf}) totaled 186 including the two species of grasses, and across this complete dataset δ^2H_{leaf} ranged between -83 and 37 ‰ with an overall mean value of 5 ± 20 ‰ (Fig. 3). The δ^2H_{leaf} of grasses (mean -2 ± 14 ‰, n = 18) were less enriched than those of trees (10 ± 16 ‰, p

250 <0.01, n = 63), while those of shrubs were intermediate (3 ± 22 ‰, p = 105). Leaf phenology had a significant effect (p <0.001) on the δ^2H_{leaf} of shrubs with values of 16 ± 7 ‰ and -4 ± 24 ‰ for deciduous (n = 38) and evergreen (n = 67) shrubs, respectively. The two evergreen shrubs of the Capparaceae family in particular showed strongly depleted δ^2H_{leaf} values, respectively -11 ± 19 ‰ for *Thylachium africanum* and -57 ± 14 ‰ for *Maerua* sp. Even if these outliers are removed from the group of evergreen shrubs, the difference in δ^2H_{leaf}

255 between deciduous and evergreen remains significant (p < 0.05). No such difference (p >0.05) was observed

between the δ^2H_{leaf} of deciduous (n = 23) and evergreen (n = 40) trees. At the lake shore, trees showed higher δ^2H_{leaf} values (9 ± 20 ‰, n = 38) than shrubs (-6 ± 20 ‰, p <0.05, n = 12). Shrubs in the savannah have higher δ^2H_{leaf} values (13 ± 13 ‰, n = 31) than those on the crater rim (0 ± 25 ‰, p <0.05, n = 62) and at the lake shore (-6 ± 20 ‰, p <0.05, n = 12), while δ^2H_{leaf} values of shrubs at the lake shore and on the crater rim did not differ

260   significantly (p >0.05). Only the shrub *Vepris uguenensis* (in both habitats) and the trees *Sideroxylon* sp. and *Lepisanthes senegalensis* (only lakeshore habitat) showed an effect of seasonality on δ^2H_{leaf}. *V. uguenensis* showed depleted δ^2H_{leaf} values during the dry season (-11 ± 16 ‰) versus enriched values during both rainy seasons (7 ± 11 ‰), whereas *Sideroxylon* sp. and *L. senegalensis* showed most depleted δ^2H_{leaf} during the long rain season (-10 ± 20 ‰ and -6 ± 11 ‰ respectively) and most enriched δ^2H_{leaf} during the short rain season (27 ±

265   3 ‰ and 33 ± 3 ‰ respectively).

4.4 Factor of deuterium enrichment from xylem to leaf water ($\epsilon_{l/x}$)

The enrichment factor $\epsilon_{l/x}$ of deuterium fractionation between xylem and leaf water could be determined on a total of 133 pairs of δ^2H_{xylem} and δ^2H_{leaf} values, and this yielded an average $\epsilon_{l/x}$ for δ^2H of 24 ± 28 ‰ across all

270   habitats, plant species (trees and shrubs only) and seasons (Fig. 6); the $\epsilon_{l/x}$ for $\delta^{18}O$ is not reported here but showed the same trends. The enrichment factor showed a significant difference (p <0.001) among the three habitats, being smaller in plants at the lake shore (7 ± 23 ‰, n = 42) than in the savannah (36 ± 19 ‰, n = 29) and crater-rim plants (30 ± 30 ‰, n = 62). Growth form had no significant effect (p >0.05) on $\epsilon_{l/x}$ with values of 27 ± 29 ‰ for shrubs (n = 80) and 19 ± 25 ‰ for trees (n = 53). Significant differences (p <0.001) were found

275   between species according to their leaf phenology with $\epsilon_{l/x}$ values of 37 ± 25 ‰ deciduous plants (n = 50) and 16 ± 27 ‰ for evergreens (n = 83). An effect of seasonality was limited and could only be observed in *Sideroxylon* sp. (p <0.05) and *Lepisanthes senegalensis* (p <0.01), reflecting the trends in δ^2H_{leaf}.

5 Discussion

5.1 Water sources: isotopic composition of precipitation, groundwater and lake water

280   Equatorial East Africa has a pronounced bimodal seasonality in rainfall, characterized by 'long rains' from March until May and 'short rains' from late October until December separated by a long dry season (Nicholson, 2000). During November-December of 2013, when Indian Ocean moisture was advected by NE monsoon winds, δ^2H_{prec} and $\delta^{18}O_{prec}$ were more depleted than during February through May 2014, when Indian Ocean moisture was advected by SE monsoon winds (Fig. 2). This result stresses the importance of the air mass trajectory in

285   controlling seasonal patterns of rainwater isotopic signatures. For the location of Lake Challa, moist air advected by the NE monsoon has travelled a longer distance overland compared to moist air advected by the SE monsoon. However within the short rain season we recorded a considerable difference between the very strongly depleted δ^2H_{prec} value for November precipitation (-48 ‰), representing the first rains after the dry season, and only modestly depleted δ^2H_{prec} value for December (-5 ‰). This indicates that not only the general air mass trajectory

290   but also other phenomena play an important role in determining the isotopic composition of monthly precipitation in any particular year. The total amount of rainfall during the study period (692 mm) was slightly above reported values for the long-term mean annual precipitation in the Challa region, which vary between 583

mm for Taveta 1989-2005 and 532 mm for Challa 2000-2007 (Fig. 2) and ~650 mm for Challa region (Hemp, 2006b). According to the Kenya Food Security Steering Group (2014), the onset of the 2013 short rain season

295 was delayed by 2-3 weeks to start in mid-November instead of late October. The short rain season also ceased in mid-December, a week earlier than normal, however the rainfall amounts in both November and December were well above average. In addition the 2014 long rain season started earlier and ceased later than normal, with monthly rainfall amounts for February and June more than double those of an average year (Fig. 2). On the other hand the month of January 2014 was exceptionally dry , and the main dry season of July through September was

300 drier than usual (Fig. 2). The $\delta^2 H_{prec}$ and $\delta^{18} O_{prec}$ of the dry month of July are clearly more enriched compared to the corresponding values for both wet periods (Fig. 2). This result illustrates the 'amount effect' (Dansgaard, 1964), which states that tropical regions characterized by limited temperature variation but strong seasonality in rainfall, show a stronger depletion of the heavy isotopes of water at higher precipitation rates.

305 The isotopic composition of the groundwater sampled at Miwaleni spring in July 2015 (-4.6 ‰ for $\delta^{18} O$ and -20.2 ‰ for $\delta^2 H$) is consistent with data of McKenzie et al. (2011) for several groundwater wells around Mt. Kilimanjaro measured in 2006.

Monthly isotopic measurements of lake-surface water revealed very little seasonal variation in $\delta^{18} O_{lake}$ and

310 $\delta^2 H_{lake}$ (Fig. 2 and 3). Our mean $\delta^2 H_{lake}$ and $\delta^{18} O_{lake}$ values (17.4 ‰ and 2.9 ‰ respectively) during 2013-2014 are very similar to those measured by McKenzie et al. (2010) in January 2006 (19.5 ‰ and 2.3 ‰), indicating that the isotopic signature of Lake Challa surface water is also stable on an inter-annual to decadal time scale. We did observe a modest seasonal trend in both $\delta^{18} O_{lake}$ and $\delta^2 H_{lake}$ with lowest (least enriched) values around August. This is counterintuitive, as evaporative enrichment is expected to be more pronounced during the dry

315 season. Although detailed assessment of this seasonal trend is outside the scope of the present study, we offer two possible explanations. First, seasonal deep circulation of the lake's water column during the cool and windy main dry season (June-September; Wolff et al., 2014) may mix the evaporating (and thus isotopically enriched) surface water with isotopically more depleted deeper water, resulting in slight depletion of the surface water. Alternatively, dry-season evaporation may be compensated by enhanced inflow of subsurface water carrying the

320 isotopic signature of the precipitation which fell on the forested slopes of Mt. Kilimanjaro during the previous rain season (Barker et al., 2011). Whatever the cause of the modest seasonal trend in lake-water $\delta^2 H_{lake}$, plants using significant amounts of lake water can be expected to show reduced seasonality in the isotopic signature of xylem water, $\delta^2 H_{xylem}$.

325 **5.2 Xylem water: spatial and seasonal partitioning of plants' water sources**

The average isotopic composition of plants' source water is reflected in the intersection points of individual xylem samples' LELs with the LMWL. Plants that rely mostly on water from isotopically depleted NE monsoon rains will exhibit relatively low $\delta^2 H_{LMWL-int}$ values, while the opposite is true for plants relying on water from the isotopically less depleted SE monsoon rains. The distance of individual $\delta^2 H_{xylem}$ values from the LMWL along

330 their LEL is proportional to the relative degree of evaporation before uptake by the plant. The higher this parameter ED, the greater the relative importance of topsoil water (which is prone to evaporation) compared to deeper soil water. In a study region experiencing a Mediterranean climate with wet winters and dry summers,

Brooks et al. (2010) found increasingly depleted $\delta^{18}O$ and δ^2H values with increasing soil depth and argued that the first and isotopically depleted autumn rains recharged the deep and withered soil, whereas water in shallow
335   soil contains water from later, more enriched precipitation. We did not measure the isotopic composition of water along a soil profile, but also in this study the first rains after the main dry season are isotopically the most depleted. Access of plants to groundwater will similarly result in isotopically depleted xylem water because this water is mainly derived from precipitation on the forests of Mt. Kilimanjaro at 1800-2800 m.a.s.l. (Payne, 1970; Hemp, 2006a).
340

Whether plants in the Lake Challa area are deciduous or evergreen produced no systematic trends in $\delta^2H_{LMWL-int}$ and evaporation distance (Figs. S1-S2) at any of the three principal plant habitats. We expected that evergreen plants would be adapted to tap water from deep sources (i.e. isotopically depleted water) allowing them to survive the long dry season, as observed by Jackson et al. (1995) in the moist lowland tropical forest of Panama.
345   On the other hand, in a dry lowland tropical forest on Hawaii, also Stratton et al. (2000) failed to find a clear difference in δ^2H_{xylem} between deciduous and evergreen plants.

At the lake shore and on the crater rim, the evaporation distance of trees was higher than that of shrubs (Fig. S2), indicating that trees use more of the topsoil water subject to evaporation. In the savannah, however, no such
350   difference between the two growth forms was found. It is generally accepted that the deeper root systems of trees compared to shrubs allow them access to deeper soil water or groundwater (Dawson, 1996). Meinzer et al. (1999) however found that smaller trees use deeper sources of soil water than larger trees, and mentioned three factors that are possibly at play. First, large trees require large amounts of nutrients to maintain their extensive crown leaf area, and the nutrient content of topsoil water is much greater than that of water taken from greater depth.
355   Second, and likely related to the first factor, large trees have a relatively more extensive horizontal root system, in order to compensate for the reduced water content of topsoil. Finally, the relatively big stem water capacity of large trees reduces peak daytime demands for soil water uptake. Goldsmith et al. (2011) agreed that soil nutrient availability can be a strong growth-limiting factor and therefore a driver of root distributions, but observed that plant species occurring in either the understory or canopy of mature and secondary forests used a similarly
360   shallow water source. In this study, the evaporation distance of plants (both trees and shrubs) at the lake shore are strikingly higher than that of plants growing in the savannah and on the crater rim (Fig. 5). This is because lake-shore plants use a substantial fraction of lake water, which has relatively enriched δ^2H_{lake} values. The higher δ^2H_{xylem} and evaporation distance of lake-shore trees compared to lake-shore shrubs indicate that former rely more heavily on lake water while the latter tap more soil water.
365

Plants in all three local habitats showed similar, very negative $\delta^2H_{LMWL-int}$ values, indicating that they relied mostly on depleted NE monsoon rains falling between October and December (Fig. 2). In all 3 habitats the $\delta^2H_{LMWL-int}$ of plants sampled in December approached the δ^2H_{prec} of November rain (-48 ‰; Fig. 4). Probably this is because these rains represent the onset of the short rain season following a distinct 4-month long dry
370   season, recharging the local soils. This is again in accordance with Brooks et al. (2010) who described how precipitation falling early in the rainy season gradually refilled an empty soil water pool. Tightly bound in small pores, this precipitation is retained in the soil through several seasons despite later inputs. Plants at the lake shore

predictably showed only a weak (statistically insignificant) seasonal trend in $\delta^2H_{LMWL\text{-}int}$ (Fig. 4). From observations it was obvious that the shore was the wettest of all three local habitats, and plants stayed verdantly

375   green in the dry season as there was plenty of water available. Plants on the crater rim and in the savannah showed lowest $\delta^2H_{LMWL\text{-}int}$ values during December, which then increased during the following months to reach their highest recorded values during the dry season in July. This indicates that the plants' water pool was replenished stepwise by the isotopically more enriched precipitation that followed from December onwards. At the top of the crater rim there is greater variability in monthly mean $\delta^2H_{LMWL\text{-}int}$ and also the compound seasonal

380   trend is more pronounced than in savannah. Probably this is because the rim is the driest location with shallow soils on bedrock, while the savannah site at the foot of the crater has deeper soils. Altogether, our data seem to confirm the 'two water world' hypothesis in which a soil-bound water pool is used by the plants while another, highly mobile pool of precipitation water contributes to streams and groundwater (Brooks et al., 2010; Goldsmith et al., 2011; Evaristo et al., 2015).

385

From all plants on the crater rim, *Euphorbia tirucalli* had the highest evaporation distance, indicating that this evergreen shrub is adapted to use the most shallow water sources in this habitat. This allows it to exploit light precipitation events ('showers') more effectively (Caldwell et al., 1998) and to coexist with other species that draw water from deeper sources. This physiology may be due to the unique combination of CAM metabolism in

390   the succulent stem of this species and C3 metabolism in its non-succulent leaves (Van Damme, 1999; Hastilestari et al., 2013). *E. tirucalli* has a high drought tolerance because under extremely dry conditions its C3 leaves wither and die (i.e., become deciduous) while the stem continues its CAM photosynthesis (Hastilestari et al., 2013). However, the succulent stem made it difficult to separate phloem and xylem, so that the collected water may be a combination of both and its isotopic signature is enriched because of transpiration.

395

5.3 Parameters affecting xylem-to-leaf deuterium enrichment

Averaging all sampled species over all seasons, the δ^2H enrichment factor $\varepsilon_{l/x}$ from xylem to leaf water in plants of the Lake Challa area is 24 ± 28 ‰. Simulating global patterns of leaf water δ^2H enrichment, Kahmen et al. (2013) predicted δ^2H-enrichment to be strong in arid biomes (40-100 ‰), intermediate in temperate biomes (10-

400   30 ‰) and weaker in humid tropical biomes (0-20 ‰). Our values for the semi-arid tropical area of Lake Challa fit between the arid and temperate models. The broad range of δ^2H_{leaf} values measured in this study (from -83 ‰ to 37 ‰) is not surprising as several environmental variables, including ambient temperature, relative humidity, wind speed and the δ^2H of atmospheric water vapor influence the leaf water deuterium enrichment (Sachse et al., 2012 and references therein). Furthermore, taking into account highly diverse leaf morphology large variations in

405   δ^2H_{leaf} between plant species are expected (Smith and Freeman, 2006; Kahmen et al., 2008). Large diurnal variations in δ^2H_{leaf} are observed as well (Cernusak et al., 2002; Li et al., 2006; Kahmen et al., 2008), which is why our samples were taken as much as possible around the same time in the day.

In our study area, leaf phenology appears to exert an important influence on δ^2H_{leaf}, and this is also reflected in

410     $\varepsilon_{l/x}$ (Fig. 6). The species-specific variation in $\varepsilon_{l/x}$ is likely explained by differences in plant physiology and biochemistry. The highest δ^2H_{leaf} and $\varepsilon_{l/x}$ values were displayed by deciduous species, at least on the crater rim and in the savannah. Evergreen plants keeping their foliage during the dry season must be protected against

drought stress by a high degree of succulence or sclerophylly (thickened or hardened leaves) to reduce moisture loss (Chabot and Hicks, 1982). Burghardt and Riederer (2003) observed that the peak cuticular transpiration rates

415 of evergreens are approximately one order of magnitude lower than those of deciduous species. Thus the adaptive traits of evergreens which reduce water loss and lower transpiration rates, result in lower xylem-to-leaf deuterium enrichment. Extremely low $\varepsilon_{l/x}$ values of respectively -24 ± 30 ‰ and 8 ± 15 ‰, indicative of very limited evapotranspiration, were recorded for *Maerua* sp. and *Thylachium africanum*, two evergreen Capparaceae growing on the crater rim (Fig. 6). Slightly depleted δ^2H_{leaf} values relative to δ^2H_{xylem} (i.e. small,

420 negative $\varepsilon_{l/x}$ values) have previously been reported for some trees in *Rhizophora* mangroves (Ladd and Sachs 2015). These authors ascribed this to the high ambient relative humidity, resulting in a small vapor pressure gradient across the leaf surface (see also Helliker and Ehleringer, 2000; Farquhar et al., 2007). The atypical $\varepsilon_{l/x}$ values of the two Capparaceae in this study are possibly associated with their xerophytic traits, in particular the waxy appearance of the leaves in many species of this family (Elffers et al., 1964). These waxy and leathery

425 leaves are useful adaptations to survive a long dry season, as plants lose water not only via their stomata but also across the cuticle (Schonherr, 1982). Oliveira et al. (2003) observed that the waxes of *Capparis yco*, a species belonging to the Capparaceae, are very efficient against water loss due to the predominance of *n*-alkanes in their composition. Thus these waxes on the leaf surfaces of the two Capparaceae species will reduce the plants' transpiration and thus possibly explain their small $\varepsilon_{l/x}$ values.

430

The $\varepsilon_{l/x}$ values of Lake Challa area plants did not show systematic variation according to growth form (that is, trees versus shrubs). Judging from Fig. 6, the plants' habitat did significantly affect $\varepsilon_{l/x}$. However, *Grewia tephrodermis*, *Vepris uguenensis* and *Thylachium africanum* show similar $\varepsilon_{l/x}$ values irrespective of the habitat in which they were sampled (Fig. 6). This suggests that the overall difference in $\varepsilon_{l/x}$ values according to habitat is

435 due to differences in the plant assemblage occurring in each habitat, rather than habitat-specific factors. Also the temporal variability in $\varepsilon_{l/x}$ was limited with only two of the sampled species (*Sideroxylon* sp. and *Lepisanthes senegalensis*) showing significant differences across seasons. Both species displayed lowest $\varepsilon_{l/x}$ during the long rain season (SE monsoon) and highest $\varepsilon_{l/x}$ during both the short rain season (NE monsoon) and the dry season. Several studies observed that stomatal conductance in savannah plants declines during the dry season due to

440 increased vapor pressure deficits and declining soil water availability (Duff et al., 1997; Prior et al., 1997). Surprisingly, O'Grady et al. (1999) detected higher transpiration rates in open-canopy eucalyptus forests in Australia during the dry season than during the wet season, mainly because of higher evaporative demand. Meinzer et al. (1993) on the other hand found similar mean transpiration rates in a lowland tropical forest tree during the wet and dry seasons despite variation in the leaf-to-air vapor pressure difference. Our data on the

445 majority of species sampled around Lake Challa are consistent with the observations of Meinzer et al. (1993) in that they do not show a significant difference in $\varepsilon_{l/x}$ among seasons. In summary, our results point to the fact that at the local scale of a single study area with several distinct plant habitats, the plant species assemblage and associated therewith the prevailing leaf phenology are the primary factors influencing xylem-to-leaf water δ^2H enrichment, while growth form and seasonality have negligible effects.

450

Along a major hydroclimate gradient influencing the composition of plant assemblages at the (sub-) continental scale, the δ^2H of plant leaf wax *n*-alkanes varies with the mean δ^2H value of local precipitation (Sachse et al.,

2004; Huang et al., 2004; Hou et al., 2008; Polissar and Freeman, 2010; Garcin et al., 2012; Tipple and Pagani, 2013); it is this relationship which underpins the use of leaf-wax δ^2H signatures in hydroclimate reconstruction.

455 Despite its enormous potential, hydroclimate interpretations remain troubled by uncertainties in the effects of past variation in water source δ^2H, xylem-to-leaf δ^2H enrichment, and the biosynthetic isotopic depletion which occurs during n-alkane synthesis (Sessions et al., 1999; Liu and Yang, 2008; Smith and Freeman, 2006; Sachse et al., 2012). This study investigated the first two of these sources of uncertainty. The third source of uncertainty requires investigations into whether the effects of growth form, phenology, habitat and seasonality that are (not)

460 reflected in $\varepsilon_{l/x}$, are preserved in the leaf wax n-alkanes.

6 Conclusions

In this study, we measured $\delta^{18}O$ and δ^2H of precipitation, lake water, groundwater and plant xylem and leaf water across different plant species, seasons and habitats with varying distances to Lake Challa in equatorial East Africa. We found that the trajectory of the air masses delivering rain to the area considerably influences the

465 seasonal signature of water isotopes in precipitation, but not all of its variability can be explained in this way. Lake-surface water showed stable $\delta^{18}O_{lake}$ and δ^2H_{lake} with seasonally lowest isotopic values during the dry season.

No statistical differences were observed between the source water of evergreen and deciduous plants in the three principal habitats around Lake Challa, as inferred from the intersection point ($\delta^2H_{LMWL-int}$) of the plants' LELs

470 with the LMWL. In all three habitats, the plants' principal source water was NE monsoon precipitation falling during the short rain season (in this year, mostly November-December), likely because these first rains following the long dry season recharged the dry soil. The plants' available water pool was replenished only stepwise by more enriched precipitation from the SE monsoon falling during the long rain season (in this year, February-May). These results are in agreement with the 'two water world' hypothesis, where plants rely on a static water

475 pool while a mobile water pool recharges groundwater and is exported to streams as run-off. The evaporation distance of trees was higher than shrubs in both the lake shore and crater rim habitat, indicating that trees use more topsoil water, presumably because the trees' root distribution is driven by their high nutrient needs to sustain a large canopy. The high evaporation distance of plants at the lake shore further indicates that these plants used a significant fraction of lake water, as expected. Based on our results, leaf phenology (deciduous versus

480 evergreen) plays a key role in determining the xylem-to-leaf water deuterium enrichment in this semi-arid tropical environment. Deciduous species gave highest $\varepsilon_{l/x}$ values, probably because evergreens are better protected against loss of moisture.

Acknowledgments

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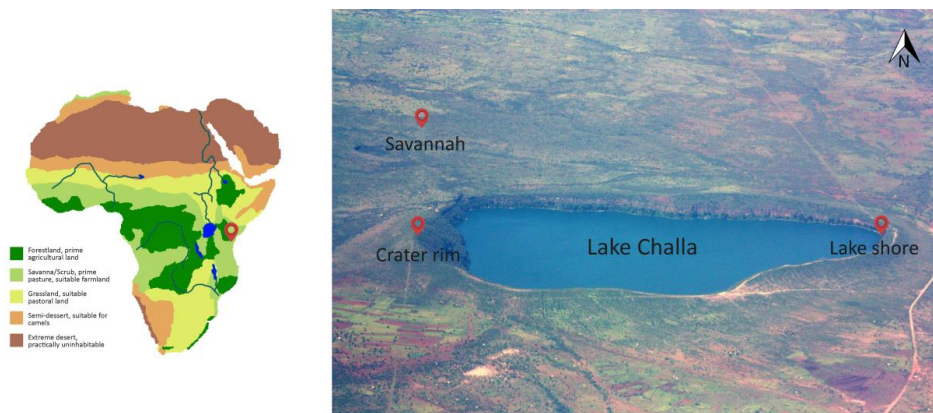
Figure 1: Lake Challa, situated in equatorial East Africa, on a continent-scale vegetation map (left) with the different sampled habitats (right). Adapted from Wikimedia Commons.

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Table 1: Studied plant species with their respective growth form, phenology and sample habitat.

| Species	Family	Growth form	Leaf phenology	Habitat
Acacia gerrardii	Leguminosae	Tree	Evergreen	Savannah
Boswellia neglecta	Burseraceae	Tree	Deciduous	Crater rim
Ficus sycomorus	Moraceae	Tree	Deciduous	Lake shore
Lepisanthes senegalensis	Sapindaceae	Tree	Evergreen	Lake shore
Sideroxylon sp.	Sapotaceae	Tree	Evergreen	Lake shore
Commiphora africana	Burseraceae	Shrub	Deciduous	Crater rim
Euphorbia tirucalli	Euphorbiaceae	Shrub	Evergreen	Crater rim
Grewia tephrodermis	Tiliaceae	Shrub	Deciduous	Savannah, crater rim
Maerua sp.	Capparaceae	Shrub	Evergreen	Crater rim
Thylachium africanum	Capparaceae	Shrub	Evergreen	Lake shore, crater rim
Vepris uguenensis	Rutaceae	Shrub	Evergreen	Savannah, crater rim
Ximenia americana	Olaceae	Shrub	Evergreen	Savannah
Enteropogon macrostachyus*	Poaceae	Grass	Perennial	Savannah
Themeda triandra*	Poaceae	Grass	Perennial	Savannah, crater rim

*Only leaf water sampled

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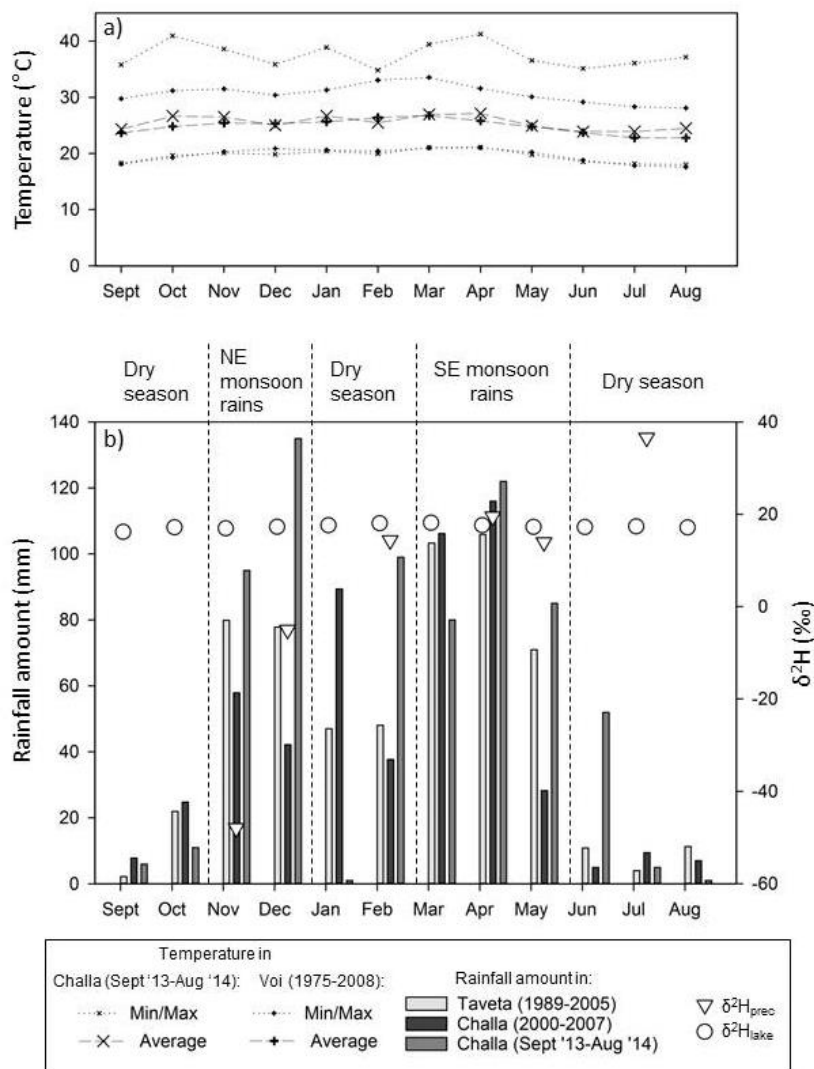
Figure 2: a) At Lake Challa, the monthly average temperature varied slightly between 23.9 °C in June-July and 27.1 °C in April. The minimum and maximum temperatures for both the study area and Voi (Kenya) are shown. **b)** Monthly rainfall distribution from September 2013 to August 2014 with the isotopic composition of precipitation and lake water (δ^2H_{prec} and δ^2H_{lake}). The total amount of rainfall during the study period (692 mm) was slightly above reported values for the long-term mean annual precipitation in the Challa region. Note that the 2014 long rain season atypically started already in February and ceased in June.

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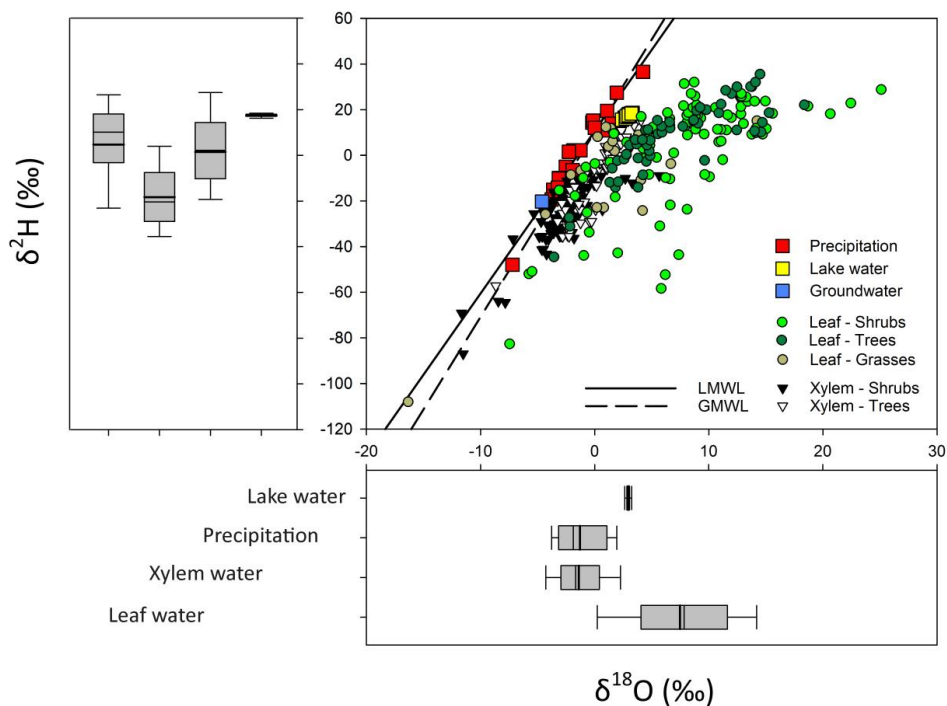
Figure 3: Xylem and leaf water δ²H and δ¹⁸O values of all plant species, seasons and plant habitats in the vicinity of Lake Challa, and lake, precipitation and groundwater against the LMWL (δ²H= 7.12*δ¹⁸O + 10.69 ‰, black line). The boxplots show the mean (bold line), minimum, first quartile, median, third quartile and maximum for the isotopic composition of leaf water, xylem water, precipitation and lake water. LMWL: local meteoric water line, GMWL: global meteoric water line.
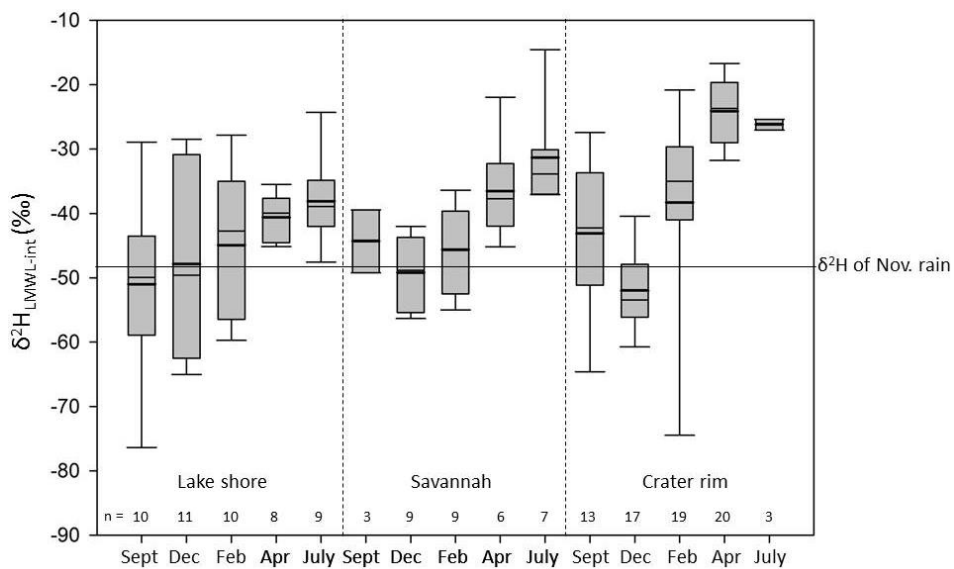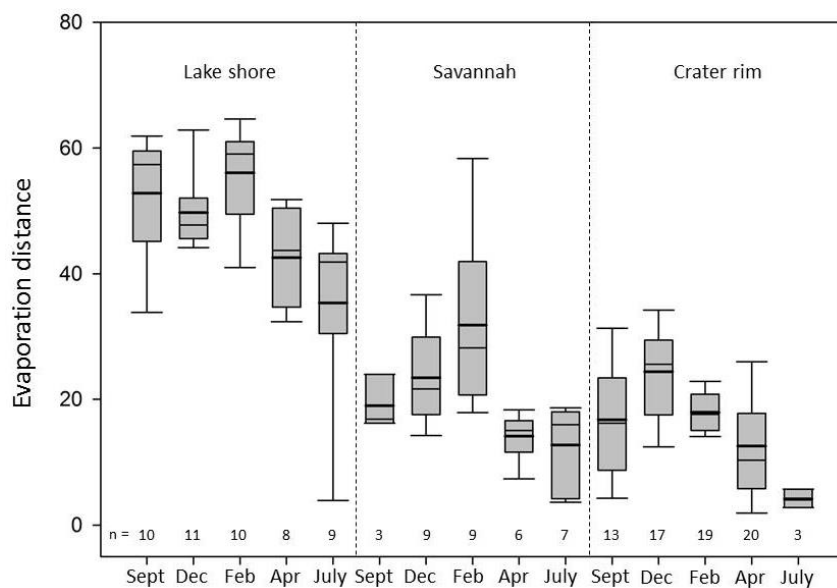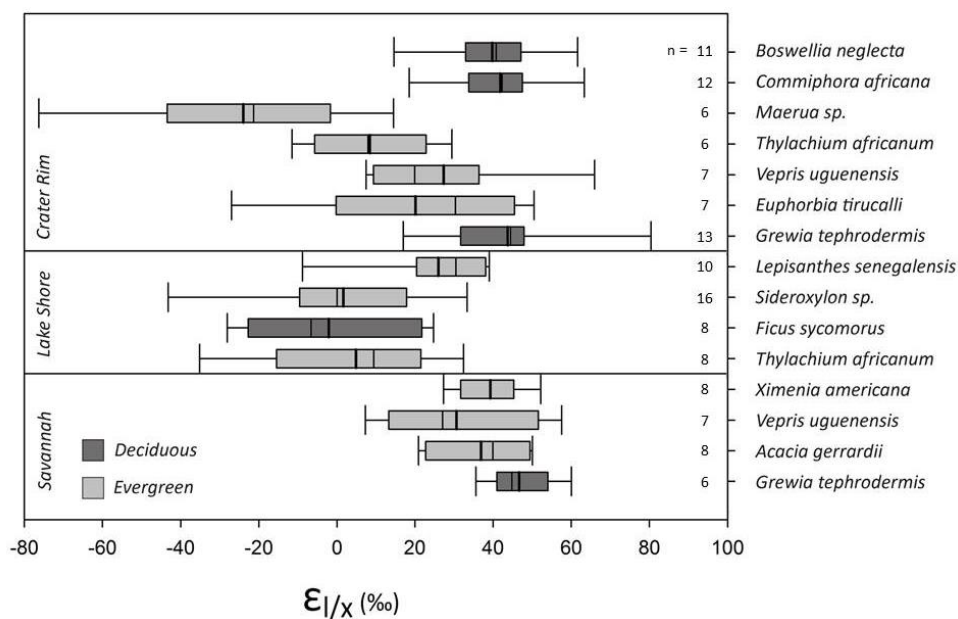
Figure 4: $\delta^2H_{LMWL-int}$ **of all sampled plant species, estimating the average isotopic signature of the source of xylem water, among habitats and seasons. Nov.: November, n: amount of samples.**
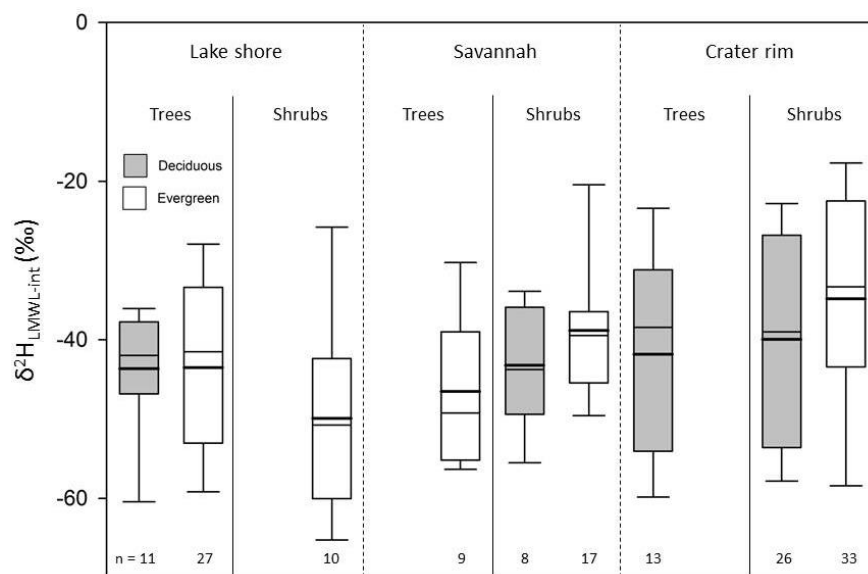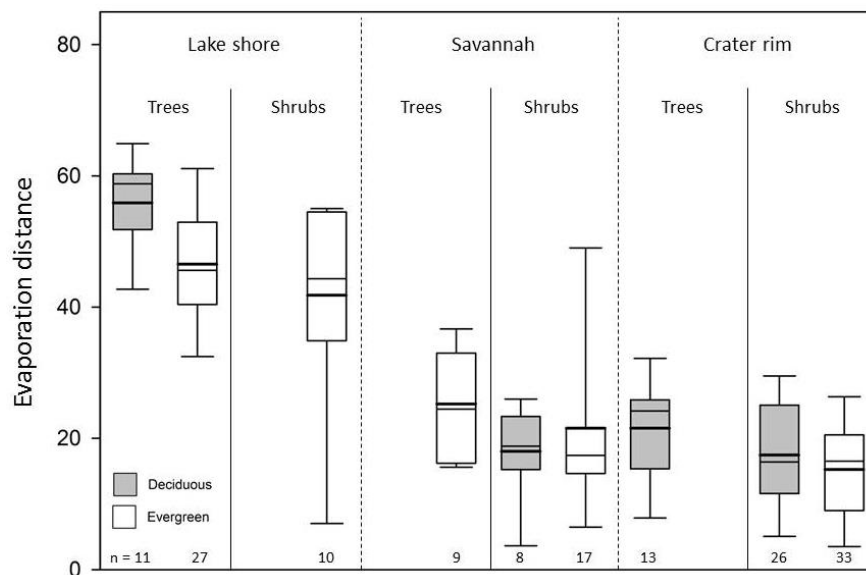

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Figure 5: The evaporation distance of xylem samples, describing the relative degree of evaporation by calculating the distance from the LMWL along an evaporation line, among habitats and seasons. n: amount of samples.

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Figure 6: δ²H enrichment factor $\varepsilon_{l/x}$, characterizing the hydrogen-isotopic fractionation between xylem and leaf water, among habitat and leaf phenology. Deciduous plants gave higher $\varepsilon_{l/x}$ than evergreens. Note that *Grewia tephrodermis*, *Vepris uguenensis* and *Thylachium africanum* showed similar $\varepsilon_{l/x}$ independent from sample habitat. n: amount of samples.

Figure S1: $\delta^2H_{LMWL-int}$ of all sampled plant species, estimating the average isotopic signature of the source of xylem water, among habitat, growth form and leaf phenology. n: amount of samples.

Figure S2: The evaporation distance of xylem samples, describing the relative degree of evaporation by calculating the distance from the LMWL along an evaporation line, among habitat, growth form and leaf phenology. n: amount of samples.

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