Leaf wax n-alkane δD values reflect the evaporative deuterium enrichment of leaf water

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Leaf water deuterium enrichment shapes leaf wax *n*-alkane δD values of angiosperm plants II: Observational evidence and global implications

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Abstract

Leaf wax *n*-alkanes are long-chain hydrocarbons that can persist in sedimentary records over geological timescales. Since their hydrogen isotopic composition (expressed as a δD value) can be correlated to the δD values of precipitation, leaf wax *n*-alkane δD values have been advocated as new and powerful proxies for paleohydrological research. The exact type of hydrological information that is recorded in the δD values of leaf wax *n*-alkanes remains, however, unclear. In a companion paper we provide experimental evidence showing that the δD values of leaf wax *n*-alkanes of angiosperm plants grown under controlled environmental conditions not only reflect δD values of precipitation – as has often been assumed – but that evaporative deuterium (D)-enrichment of leaf water has an additional critical effect on their δD values. Here we present a detailed observational study that illustrates that evaporative D-enrichment of leaf water also affects the δD values of leaf wax *n*-alkanes in plants from natural ecosystems along a 1500 km climate gradient in Northern Australia. Based on global simulations of leaf water D-enrichment we show that the effects of evaporative D-enrichment of leaf water on leaf wax *n*-alkane δD values is relevant in all biomes but that it is particularly important in arid environments. Given the combined influence of precipitation δD values and leaf water D-enrichment we argue that leaf wax *n*-alkane δD values contain an integrated signal that can provide general hydrological information, e.g. on the aridity of a catchment area. We also suggest that more specific hydrological information and even plant physiological information can be obtained from leaf wax *n*-alkanes if additional indicators are available to constrain the plant- and precipitation-derived influences on their δD values. As such, our findings have important implications for the interpretation of leaf wax *n*-alkane δD values from paleohydrological records. In addition, our investigations open the door to employ δD values of leaf wax *n*-alkanes as new ecohydrological proxies in contemporary plant and ecosystem sciences.

1. INTRODUCTION

Leaf wax *n*-alkanes are long-chain hydrocarbons with 25–35 carbon atoms that are vital components of the waxy cuticle that covers the leaves of terrestrial plants (Eglinton and Hamilton, 1967). Leaf wax *n*-alkanes are present in soils, sediments and even in atmospheric dust and can persist in the environment over millions of years (Eglinton and Eglinton, 2008). In addition, the δD values of leaf wax *n*-alkanes obtained from lake surface sediments and soils show tight correlations with δD values of precipitation (Sachse et al., 2004; Hou et al., 2008; Rao et al., 2009; Polissar

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and Freeman, 2010; Luo et al., 2011; Garcin et al., 2012). With this exceptional combination of properties, leaf wax *n*-alkanes and their δD values have been suggested as powerful new proxies that can provide important hydrological information for a range of scientific disciplines, including paleohydrology, ecosystem sciences and forensic research (e.g., Schefuß et al., 2005, 2011; Pagani et al., 2006; Tierney et al., 2008).

The primary control of leaf wax *n*-alkane δD values is the isotopic composition of the plant's source water (soil water recharged by precipitation) (Sachse et al., 2012). Several observational studies have, however, suggested that deuterium (D)-enriched leaf water (ΔD) can also affect the δD values of leaf wax *n*-alkanes (Sachse et al., 2004, 2009, 2010; Smith and Freeman, 2006; Pedentchouk et al., 2008; Feakins and Sessions, 2010; Polissar and Freeman, 2010). Leaf water can become enriched in D as a result of evaporative water loss from the leaf and equilibrium exchange of hydrogen isotopes between the leaf and the surrounding water vapor (Cernusak et al., 2005; Barbour, 2007; Farquhar et al., 2007; Kahmen et al., 2011a). If this D-enrichment is translated into the δD values of *n*-alkanes, their δD values will not simply reflect hydrological signals derived from the δD of precipitation, but will also reflect plant physiological processes such as evapotranspiration. An effect of D-enriched leaf water on the δD values of leaf wax *n*-alkanes would thus have important implications for the interpretation of leaf wax *n*-alkane δD values when these are applied as (paleo)hydrological proxies (Sachse et al., 2012).

Despite their relevance, the magnitude of the effect of D-enriched leaf water on the δD values of leaf wax *n*-alkanes has remained controversial. In a companion paper (Kahmen et al., 2013) we report the results of an experimental study, in which we quantified the effects of D-enriched leaf water on the δD values of leaf wax *n*-alkanes for five different angiosperm plant species from different phylogenetic groups that we grew in climate-controlled growth chambers. Our experiment revealed that for the investigated dicotyledonous plant species the full extent of leaf water evaporative D-enrichment is recorded in leaf wax *n*-alkane δD values. For monocotyledonous plants (grasses) the experiment suggested that between 18% and 64% of the D-enrichment in leaf water was recorded in the δD values of their *n*-alkanes. This experimental study thus provided strong evidence for an important effect of leaf water evaporative D-enrichment on the δD values of leaf wax *n*-alkanes under controlled environmental conditions. What remains unclear is whether the effect of D-enriched leaf water that we observed under experimental conditions is also relevant to the δD values of leaf wax *n*-alkanes under natural conditions, where D-enrichment of leaf water can vary substantially across several spatial and temporal scales and may not be large enough to be a possible important source of variation for leaf wax *n*-alkane δD values (Hou et al., 2008).

To resolve these uncertainties, we report here the results of a detailed observational study, where we tested if the influence of leaf water evaporative D-enrichment on the δD values of leaf wax *n*-alkanes that we determined under experimental conditions is also relevant for plants that grow in different natural ecosystems. We assessed this potential effect for several *Eucalyptus* and *Acacia* species from different ecosystems that ranged from arid shrublands to tropical savanna along a 1500 km climate gradient in Northern Australia. We also employed an isoscape model to estimate global patterns of leaf water D-enrichment and to determine how D-enriched leaf water could affect the δD values of leaf wax *n*-alkanes in different biomes across the planet. The work that we present here is thus intended not only to corroborate the experimental work that we report in the companion paper but also to provide a mechanistic basis for the interpretation of leaf wax *n*-alkane δD values when these are applied as (paleo)hydrological proxies worldwide.

2. MATERIALS AND METHODS

2.1. Description of the North Australian climate gradient

To determine the influence of D-enriched leaf water on leaf wax *n*-alkane δD values under natural conditions, we sampled and analyzed xylem water, leaf water, soil water, water vapor, and leaf wax *n*-alkanes for their δD values along a transect that spans 1500 km from Alice Springs to Darwin in the Northern Territory in Australia (Schulze et al., 1998). The transect proved ideal for our investigation, as the δD values of precipitation – i.e. the plants' source water – showed no distinct trend along the transect (Bowen and Wilkinson, 2002). In contrast, precipitation amount increases and the evaporative demand of the atmosphere decreases substantially from the arid regions around Alice Springs at the southern end of the transect to the tropical regions around Darwin in the north (Table 1, Fig. 1). The vegetation along the climate gradient follows a gradual transition from arid shrubland in the south to tropical savanna in the north and consists mainly of species belonging to the two dominant Australian genera *Acacia* and *Eucalyptus*.

2.2. Leaf water, xylem water, soil water, and water vapor sampling along the transect

We selected six sampling sites along the climate gradient that become more arid from the north to the south (Table 1). The sites were located at sufficient distance from any roads to avoid disturbance or contamination of the vegetation by vehicle exhaust. We visited each site one day in April and one day in September 2010 and sampled fresh leaves and xylem water of one *Acacia* and one *Eucalyptus* species at each site. For each species we sampled three individual trees per site that served as replicates. The same individual trees were sampled in April and September. The names of the species at each site are given in Table 1. No *Eucalyptus* plants were found at the Barrow Creek site and no *Acacia* plants at the Katherine site so that we sampled only one species (either *Acacia* or *Eucalyptus*) at these sites.

For the Eucalypt group, we not only collected samples from the genus *Eucalyptus* sensu-stricta but also of the genus *Corymbia*. Species of the genus *Corymbia* are phylogenetically closely related to species of the genus *Eucalyptus*. In

Table 1
Description of the six sites of the North Australian climate gradient. Relative humidity, air temperature and precipitation are mean annual values for 2010. Climate data are kindly provided by the Australian Bureau of Meteorology (http://www.bom.gov.au). Species of the genus *Corymbia* are phylogenetically closely related to species of the genus *Eucalyptus*. We therefore consider species of the genus *Corymbia* as members of the 'Eucalyptus' group throughout the manuscript.

Location	Darwin	Katherine	Elliott	Tennant creek	Barrow creek	Alice springs
Southern latitude (°)	12.43	14.47	17.50	19.65	21.52	23.70
Longitude (°)	130.88	132.36	133.51	134.16	133.90	133.83
Elevation (masl)	33	143	234	365	507	598
Relative humidity 9 am (%)	71.2	66.4	49.3	39.3	38.8	42.1
Relative humidity 3 pm (%)	53.4	38.3	30.6	24.7	24.4	25.1
Air temperature 9 am (°C)	26.7	25.6	25.3	24.1	22.1	20.5
Air temperature 3 pm (°C)	30.8	33.1	33.4	30.9	29.5	27.8
Precipitation (mm)	1705	1119	596	455	319	278
Eucalyptus	*Eucalyptus miniata*	*Corymbia foelscheana*	*Corymbia terminalis*	*Eucalyptus pruinosa*	–	*Corymbia terminalis*
Acacia	*Acacia auriculiformis*	–	*Acacia colei*	*Acacia cowleana*	*Acacia kempiana*	*Acacia kempiana*

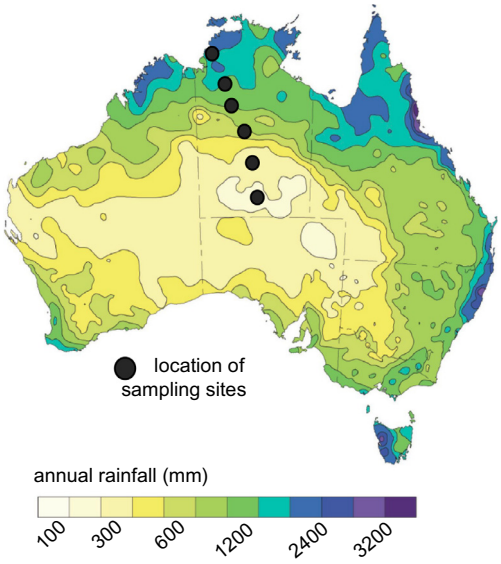

Fig. 1. Position of the six sampling sites and the variation in mean annual precipitation along the gradient in the Northern Territories in Australia. The precipitation map of Australia was kindly provided by the Australian Bureau of Meteorology (http://www.bom.gov.au).

fact, the genus *Corymbia* has only recently been separated from the genus *Eucalyptus* and both genera are often referred to as 'Eucalypts' and are morphologically and ecologically not distinct (Parra-O et al., 2006). We therefore consider species of the genus *Corymbia* as members of the '*Eucalyptus*' group throughout the manuscript.

To determine the isotope composition of leaf water we collected 3 – 10 leaves and merged these to one bulk sample per replicate tree. Leaves were randomly collected from the canopy of trees to provide samples that were representative for the entire canopy. To characterize the diurnal pattern of leaf water δD values, leaves were collected at 8:00, 11:00, 14:00, 17:00 h. All harvested *Eucalyptus* leaves had their mid-vein removed and the leaf lamina was stored in 10 ml exetainer vials. The *Acacias* that we sampled had phyllodes (rather than true leaves) that lacked a distinct midrib. For the *Acacias*, we sampled the whole phyllode. The vials were kept frozen until leaf water extractions (see below). To determine the δD values of xylem water (i.e. the plant's source water), we sampled 1–3 twigs that were randomly chosen from the canopy and merged these to one bulk sample for each replicate tree. Twigs were collected once in the afternoon and were 0.5–1.0 cm in diameter. Immediately after the twigs were collected we removed all phloem tissue with a swiss army knife and stored the xylem tissue in 10 ml exetainer vials. The vials were kept frozen until xylem water extraction (see below).

Soil for soil water δD analysis was collected from 10 cm, 30 cm, 50 cm and 80 cm soil depth at each site in April and September (except the Barrow Creek site where no soil samples were collected). Four replicate soil pits with 10 cm diameter were drilled at each site using a petrol-powered drill. Replicate soil pits were spaced at least 5 m apart in a site. Soil was brought to the surface with the drill and immediately transferred to 10 ml exetainer vials, which were kept frozen until soil water extraction (see below).

We collected atmospheric vapor for δD analysis at each site except Barrow Creek several times throughout the day that we visited the site in April and September. Vapor was collected using a self-assembled cryo-trap. Air was pumped at a flow rate of 10 l/h trough a 1 m long PE tube (inner diameter 5 mm) that was coiled three times and the coils submerged in a dry ice/ethanol slush (−80 °C) to form a loop trap. After vapor collection, the loop traps were sealed, the frozen water thawed and transferred into 2 ml GC cap vials with Teflon sealed screw caps. The cryo-trap was tested in the lab prior to field work, showing that the device traps vapor at sufficient amounts (>99%) for isotope

analysis and that no fractionations occur during trapping (Kevin Simonin, unpublished data).

2.3. Water extractions and stable hydrogen isotope analysis

Leaf water, xylem water and soil water were cryogenically extracted using a method described by West et al. (2006). In brief, leaf-, xylem- and soil- samples were heated to 95 °C in their exetainer vials. The evaporated water was collected in glass U-tubes that were submerged in liquid nitrogen. All water samples were analyzed for their hydrogen isotope composition using the high-temperature carbon reduction method by coupling a high-temperature elemental analyser (TC/EA; Finnigan MAT, Bremen, Germany) to a Delta^{plus}XP isotope ratio mass spectrometer via a ConFlo III interface (Finnigan MAT, Germany (Werner et al., 1999)) at the Institute for Agricultural Sciences, ETH Zurich, Switzerland. The precision of the lab internal standard was 0.5‰ during the analysis of the data that are reported here. A detailed description of the cryogenic water extractions as well as the stable hydrogen isotope analysis is provided in the companion paper (Kahmen et al., 2013).

2.4. Modeling seasonal leaf water δD values

Individual leaf water δD values vary strongly in response to daily fluctuations in climate, atmospheric vapor δD values and leaf physiology. We therefore used the basic Craig–Gordon model (Craig and Gordon, 1965; Dongmann et al., 1974; Farquhar and Lloyd, 1993) forced with mean midday growing season meteorological data to estimate average midday leaf water δD values along the transect for October–November–December, which is the time when most leaf development occurs for plants along the transect and leaf wax *n*-alkane δD values are most likely established (Kahmen et al., 2011b):

$$\delta D_{LW} = \delta D_{SW} + \varepsilon^{+} + \varepsilon_k + (\delta D_{WV} - \delta D_{SW} - \varepsilon_k)\frac{e_a}{e_i} \qquad (1)$$

In the model, δD_{LW} is the isotope composition of leaf water, δD_{SW} is the hydrogen isotope composition of the plant's source or xylem water, ε^{+} is the equilibrium fractionation between liquid water and vapor at the air–water interfaces (Bottinga and Craig, 1969), ε_k is the kinetic fractionation that occurs during water vapor diffusion from the leaf intercellular air space to the atmosphere (Cappa et al., 2003), δD_{WV} describes the hydrogen isotope composition of water vapor in the atmosphere, and e_a/e_i is the ratio of ambient to intercellular vapor pressures (Craig and Gordon, 1965).

Before simulating mean growing season values, we first tested the model performance by simulating diurnal leaf water δD values and comparing the simulated values to our measured diurnal leaf water δD values for *Eucalyptus* and *Acacia* species along the transect in April and September. For the diurnal simulations, we used diurnal leaf physiological data (leaf temperature and stomatal conductance) and climate data (air temperature and relative humidity) that we measured along the transect in the April and September campaigns using a LiCor 1600 (LiCor Inc., Lincoln, Nebraska, USA). We also used the atmospheric vapor δD values and xylem water δD values that we determined in our April and September campaigns as described above.

After we had tested the performance of the model with our diurnal data, we simulated mean growing season midday leaf water δD values using long-term mean midday air temperature and relative humidity data for October, November, and December as input variables that were kindly provided by the Australian Bureau of Meteorology (http://www.bom.gov.au). Since we found that our measured diurnal leaf temperature values were on average not significantly different from diurnal air temperature along the transect, we used mean midday air temperature for October, November, and December as a surrogate for mean midday leaf temperature in the model. For the remaining leaf physiological and isotope data that were necessary for the simulation as input variables we used means of the data that we assessed along the transect in September as best estimates of their growing season means. For stomatal conductance we used the mean values that we determined for a species in our September campaign at a site. For source water δD values, we used the mean xylem δD values that we determined for each plant species in September along the gradient. For water vapor δD values we used the mean water vapor δD values that we measured in September at each site along the transect.

2.5. *n*-Alkane extractions, identification, quantification and isotope analysis

For *n*-alkane δD analysis we collected 10–100 leaves randomly from each replicate tree (depending on leaf size) and merged these to one bulk sample. Samples were collected in April 2010 at the end of the wet season. Leaves were stored in paper bags and dried at 60 °C for 48 h in a drying oven. After drying, leaves were ground to a fine powder using a ball mill (Retsch, Düsseldorf, Germany). Total lipid extraction and subsequent *n*-alkane purification, compound identification and quantification was carried out in the organic-geochemical lab at University of Potsdam, following the same protocols as described in the companion paper (Kahmen et al., 2013).

Hydrogen isotope compositions of *n*-alkanes were analyzed on a Trace GC coupled via a pyrolysis reactor to a MAT 253 mass-spectrometer at MARUM, University of Bremen, Germany. The H_3-factor of the instrument varied from 5.30 to 5.37 over the measuring period (5.34 ± 0.02). Isotope values were measured against calibrated H_2 reference gas and δD values are reported in ‰ V-SMOW. Since the isotopic composition of the reference gas (vs. V-SMOW) was known, we calculated δD values vs. VSMOW for the individual peaks. An *n*-alkane standard (Mix A3 from A. Schimmelmann) of 15 externally calibrated *n*-alkanes was measured in triplicate every three samples. Since the slope and intercept of the relationships between known standard and measured values were stable over the measurement period and on average 0.996 ± 0.003 and 4.9 ± 1.5, respectively (with $n = 11$), we decided against further correcting the data. All samples were run in triplicates. Additionally, we analyzed the δD values of the internal standard (5α-andro-

stane, added for quantification purposes) to monitor the precision of the analysis. The internal standard had a standard deviation of ±1.7 ($n = 34$) throughout our analysis.

2.6. Data analysis

To quantify the effect of evaporative D-enriched leaf water on the δD values of leaf wax *n*-alkanes in the different locations along the gradient, we followed a similar procedure as in our companion paper. We build our analysis on a conceptual model, where we assume that the δD values of leaf wax *n*-alkanes are determined by the δD values of a biosynthetic water pool in the leaf and the biosynthetic fractionation (ε_{bio}). The biosynthetic water pool is determined by a mix of the plants' source water and D-enriched leaf water. Importantly, this pool is not a physical reservoir but rather a conceptual pool, which contains all hydrogen available for lipid synthesis. ε_{bio} is the result of numerous individual fractionation steps during biosynthesis and results in D-depletion of lipids (Sachse et al., 2012). Although studies that directly estimated ε_{bio} are sparse, it is believed to be rather constant, at least for a given species (Zhang and Sachs, 2007). Therefore changes in the biosynthetic water pool δD values should be the driving factor for changes in *n*-alkane δD values for a given species. The magnitude of the effect of D-enriched leaf water on the δD values of leaf wax *n*-alkanes can thus be determined by estimating the contribution of leaf water to the biosynthetic water pool.

To quantify the contribution of leaf water to the biosynthetic water pool for *Acacia* and *Eucalyptus* along the transect we first determined a concentration-weighted mean leaf wax *n*-alkane δD value ($\delta D_{n\text{-ALK}}$) for each species at a site by calculating:

$$\delta D_{n\text{-ALK}} = \sum_{k=25}^{33} \frac{(\delta D_k * conc._k)}{conc._{tot}} \quad (2)$$

where δD_k are $\delta D_{n\text{-C25}} - \delta D_{n\text{-C33}}$, $conc._k$ are the concentration of $n\text{-C}_{25} - n\text{-C}_{33}$ alkanes in μg per g leaf material and $conc._{tot}$ is the total *n*-alkane concentration in μg per g leaf material.

We then determined the apparent fractionation (ε_{app}) between $\delta D_{n\text{-ALK}}$ and plant source water, which in our case equals xylem water (δD_{XY}):

$$\varepsilon_{app} = \frac{(\delta D_{n\text{-ALK}} + 1)}{(\delta D_{XY} + 1)} - 1 \quad (3)$$

Since delta values are typically reported in per mil, this implies a factor of 1000 in the equation. ε_{app} is a measure of the deviation of leaf wax *n*-alkane δD values from source water δD values and is influenced by the isotopic composition of the biosynthetic water pool and the net biosynthetic fractionation, ε_{bio}. Under the assumption of a constant ε_{bio} any variations in ε_{app} along the gradient should be caused by variations in the δD values of the biosynthetic water pool. If related to mean growing season leaf water D-enrichment above source water (ΔD, Eq. (4)), the slope of the relationship between leaf water ΔD and ε_{app} can be thus used to estimate the extent by which D-enriched leaf water contributes to the biosynthetic water pool and the intercept of the regression can be used to estimate values for the biosynthetic fractionation. ΔD was calculated as

$$\Delta_D = \frac{(\delta D_{LW} + 1)}{(\delta D_{XY} + 1)} - 1 \quad (4)$$

2.7. Global leaf water isoscape

The global distributions of northern and southern hemisphere spring (Apr–May–Jun and Oct–Nov–Dec, respectively) leaf water D-enrichment above source water (ΔD) were estimated following procedures outlined in West et al. (2008) for leaf water δD, with an updated precipitation δD dataset and modeling algorithm (http://www.waterisotopes.org; downloaded October 18, 2011) and high resolution climate data layers provided by the Climate Research Unit (CRU CL 2.0, http://www.cru.uea.ac.uk/; New et al., 2002). A steady-state, modified Craig–Gordon model of leaf water δD (see Eq. (1)) was executed in a raster modeling environment (ArcGIS 10.0, ESRI Inc., Redlands, CA). Seasonal climate grids drive the modeled D-enrichment (Apr–May–Jun and Oct–Nov–Dec for the same years) with a set of model parameters that derive physiological parameters (e.g., leaf temperature and stomatal conductance) and other drivers (e.g., barometric pressure, vapor δD) from the input grids and known or estimated biophysical processes. Plant source water δD was estimated as the long-term annual average precipitation δD and climate drivers were long-term seasonal averages for Apr–May–Jun and Oct–Nov–Dec (1961–1990) from the CRU climate grids. Model predictions were not made for grid cells/months with mean monthly temperatures less than 0 °C.

Since the isotopic composition of soil moisture is an integration of long-term precipitation inputs and the effects of soil evaporation and non-fractionating transpiration losses, this is potentially an important source of error in accurately modeling leaf water isotope ratios. While we do not resolve this issue here, leaf water ΔD (as opposed to δD) reflects the role of climate and plant physiology on leaf water evaporative D-enrichment, rather than variations associated with plant source water. We therefore take long-term annual average precipitation δD as a reasonable proxy for soil moisture δD and report leaf water D-enrichment over this source water.

Primary uncertainties associated with the leaf water ΔD surface are those associated with the interpolation algorithms used to generate the input data layers and those associated with the steady-state model employed. The underlying mechanisms of leaf water enrichment remain the subject of some debate (Barbour, 2007; Ferrio et al., 2009; Kahmen et al., 2009). We believe, however, that the model used is appropriate for the purpose of this study since organic compounds such as *n*-alkanes in leaves are produced at times when leaf water is likely to be near steady state and do not attempt to resolve uncertainties associated with spatial gradients within leaves or other potential sources of model error. We cannot currently quantitatively assess the sensitivity of the estimation to the underlying

uncertainties in either source water or climate drivers at this scale. In addition, existing data on leaf water δD at the global scale are currently insufficient for an effective model validation. However, some quantitative comments can be made regarding the uncertainties of the input data layers.

Those associated with the precipitation δD isoscape are related to the distribution of input data and the resulting model fit and geostatistical errors. The model structure followed generally that described in Bowen and Revenaugh (2003), and the 95% confidence intervals range from approximately 0.3‰ to 27‰ for the relevant spatial domains, with significant spatial structure related in part to the distribution of station locations. The uncertainties associated with the climate grids are reported in New et al. (2002). Briefly, for temperature, the reported square root of the generalized cross validation (RTGCV) was approximately 1 °C and mean daily temperature RTGCV ranged from 1 to 4 °C for large spatial domains (continents) and for relative humidity the RTGCV was approximately 3–7%.

3. RESULTS

3.1. Soil-, xylem- and leaf- water δD values along the climate gradient

We found no systematic trend in soil water δD values along the transect which is consistent with interpolated isoscapes for water isotopes in precipitation of Australia (Bowen and Wilkinson, 2002) (Fig. 2). Within sites, soil water showed distinct vertical gradients in δD (except for Darwin and Katherine in April), with more D-enriched soil water in the upper soil layers (Fig. 2). These vertical patterns were particularly evident in September (the end of the southern hemisphere winter and dry season) when soil water in the upper soil layers was substantially enriched in D in all sites.

The δD values of xylem water, i.e. the plants' source water, showed no trend of increasing or declining values along the transect (Fig. 3), which was consistent with the general patterns we had observed for soil water δD values along the transect. Xylem water was generally depleted in D compared to soil water (Fig. 2) and we detected no systematic differences in xylem water δD values collected in April or September (means across all species are −60‰ ± 8 stdev vs. −64‰ ± 11 stdev, respectively) or between xylem water δD values for *Eucalyptus* and *Acacia* plants (Fig. 3).

Across all five sites and both seasons, water vapor δD values ranged between −64‰ and −159‰. Water vapor δD values were highly variable among sites and showed no distinct pattern along the transect (Fig. 3). We found slight seasonal differences in water vapor δD values, where April values were on average more depleted in D (−113‰ ± 36 stdev) and September values were more enriched in D (−87‰ ± 12 stdev).

Diurnally measured leaf water δD values ($\delta D_{measured}$) did not differ between *Eucalyptus* and *Acacia* species and there was no evidence of species-specific D-enrichment patterns along the transect (Fig. 3). In general, average leaf water of both species was more D-enriched in September compared to April (5‰ ± 7 stdev vs. −26‰ ± 25 stdev, respectively). Although the Craig–Gordon model slightly overpredicted leaf water δD values by 6‰, the predicted diurnal leaf water δD values were in close agreement to the measured diurnal leaf water δD values (Fig. 4). Model-predicted mean growing season midday leaf water δD values showed a strong trend along the transect with values increasing from the tropical north to the arid south (Fig. 5). The D-enrichment of leaf water above source water (ΔD) of these model predicted values increased by 41‰ along the transect from 44‰ in the north to 85‰ in the south. This pattern was consistent for the two genera, which showed no significant differences in mean growing season midday leaf water δD or ΔD values.

3.2. Leaf wax *n*-alkane δD values along the transect

Leaves of *Eucalyptus* and *Acacia* species contained *n*-C_{25}, *n*-C_{27}, *n*-C_{29}, *n*-C_{31}, and *n*-C_{33} alkanes (Table 2). Individual *n*-alkane δD values of the different species that we sampled along the transect are presented in Table 3. Concentration-weighted mean *n*-alkane δD values varied between −135‰ and −182‰ for *Eucalyptus* and −82‰ and −118‰ for *Acacia* and increased along the transect for both genera consistently from the tropical north to the arid south (Fig. 5).

To assess the degree to which evaporative D-enrichment of leaf water influences the δD values of leaf wax *n*-alkanes, we assessed the contribution of D-enriched leaf water to the biosynthetic water pool (which ultimately provides the hydrogen for leaf wax *n*-alkane synthesis). We did this by relating the modeled mean growing season leaf water ΔD values to their corresponding values for ε_{app} of the individual genera along the transect. The analysis revealed slopes of 1.09 for *Eucalyptus* and 1.25 for *Acacia*, which suggests that D-enriched leaf water contributes 109 and 125% to the biosynthetic water pool in *Eucalyptus* and *Acacia*, respectively (Fig. 6).

3.3. Global patterns of leaf water D-enrichment

Globally, our model simulation predicted midday evaporative D-enrichment of leaf water for all parts of the planet, but with strong spatial variability (Fig. 7). The predicted D-enrichment of leaf water was strongest in arid biomes (40–100‰), intermediate in temperate biomes (10–30‰) and least pronounced in tropical biomes (0–20‰). These patterns were largely consistent for both, northern and southern hemisphere growing season although small regional deviations exist (Fig. 7).

4. DISCUSSION

4.1. Variability of soil-, xylem- and leaf-water δD values along the transect

Water in the upper soil layers was significantly enriched in D along the North Australia transect, in particular at the end of the dry season in September (Fig. 2). Xylem water δD values of *Eucalyptus* and *Acacia* plants were, however, consistently more negative than surface soil water

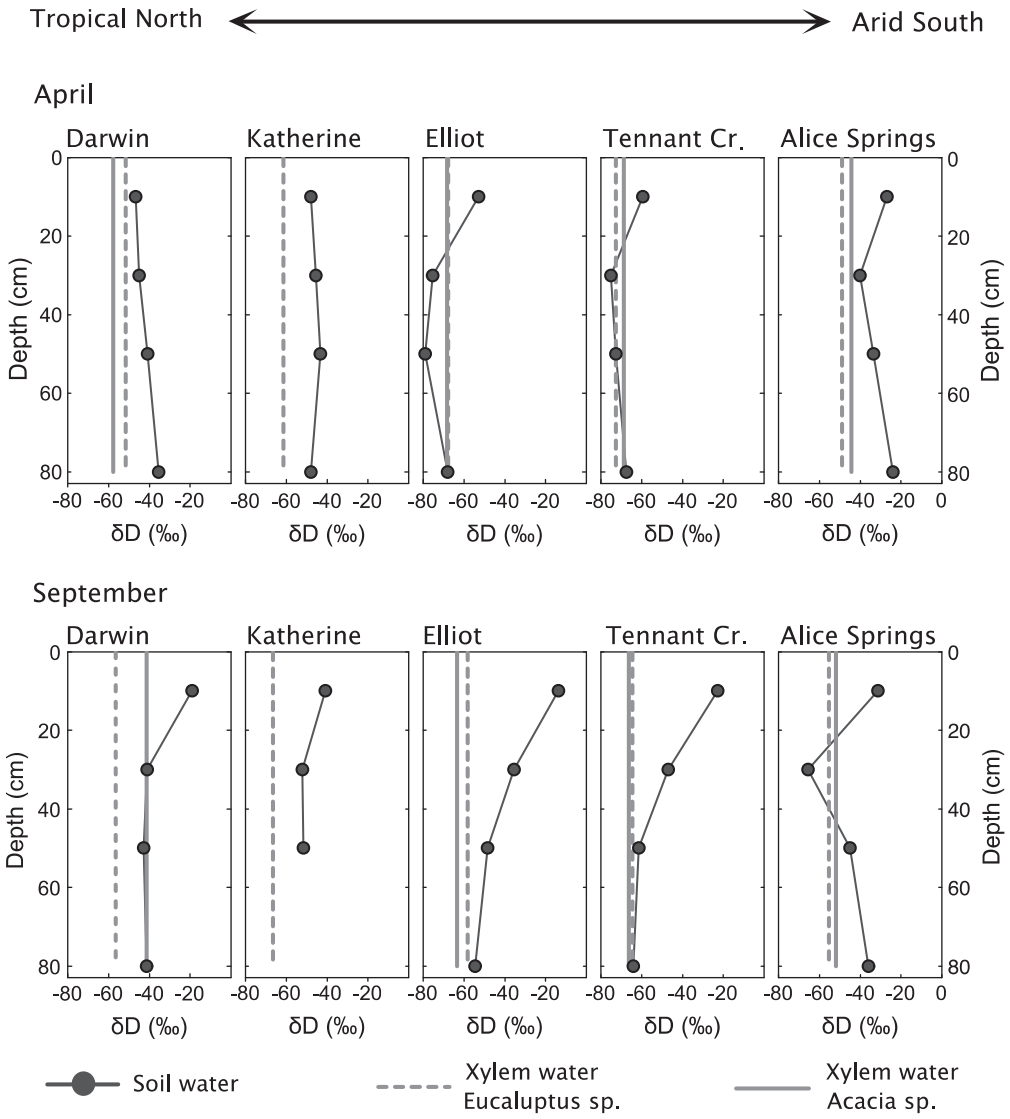

Fig. 2. Xylem water δD values for *Eucalyptus* and *Acacia* plants and vertical distribution of soil water δD values for two seasons along the North Australia Transect. Values for soil water are means of four replicate samples per site. Values for xylem water are means of three replicate plants per site. Standard deviations from the mean for soil water were always below 14‰ and are thus not displayed. Standard deviations from the mean for xylem water were always below 6‰ and are thus not displayed.

(Fig. 2). This suggests that evaporative D-enrichment of soil water did not affect the source water of *Eucalyptus* or *Acacia* but that these species access deeper water sources to meet their water demand. Our finding is in accordance with earlier studies that suggested that woody plants in arid and semi-arid environments utilized subsurface-water sources (Jackson et al., 1996; Arndt et al., 2004) and are not affected by evaporative D-enrichment of soil surface water (Feakins and Sessions, 2010).

We also found no difference in xylem water δD values for the plants between September and April (Figs. 2 and 3). This suggests that the stable hydrogen isotope composition of the plant's source water does not follow any significant seasonal variations throughout the year. It supports our previous conclusion that plants along the transect have access to a consistent water supply throughout the year and are independent of variable moisture supplies at the soil surface.

Measured diurnal leaf water δD values showed no distinct D-enrichment pattern along the climate gradient (Fig. 3). Leaf water D-enrichment is driven by a whole suite of environmental variables and can vary substantially from day to day, depending on environmental conditions (Farquhar and Cernusak, 2005; Kahmen et al., 2008). Single "point-in-time" measurements of leaf water δD values (as our diurnal measurements) are thus unlikely to give a representative impression of the typical leaf water δD values for a given site. Such representative values were, however, necessary for our effort to relate leaf water evaporative D-enrichment to the δD values of leaf wax *n*-alkanes. For our analysis we therefore choose to employ the Craig–Gordon model to predict mean growing season leaf

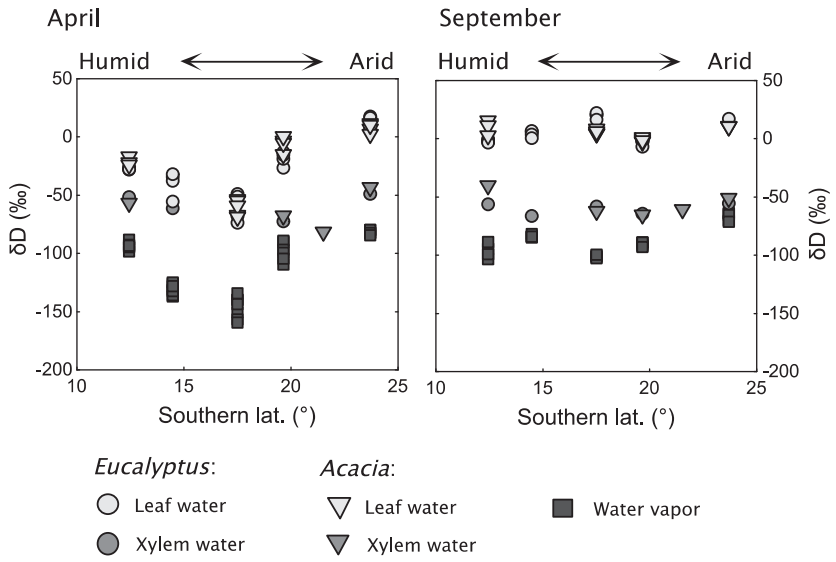

Fig. 3. Water vapor, xylem water and leaf water δD values for *Eucalyptus* and *Acacia* plants in April and September along the North Australia transect. Values for leaf water and xylem water are means of four replicate samples per species site. Leaf water was collected four times per species on the sampling days in April and September, xylem water was collected only once for each species in April and September.

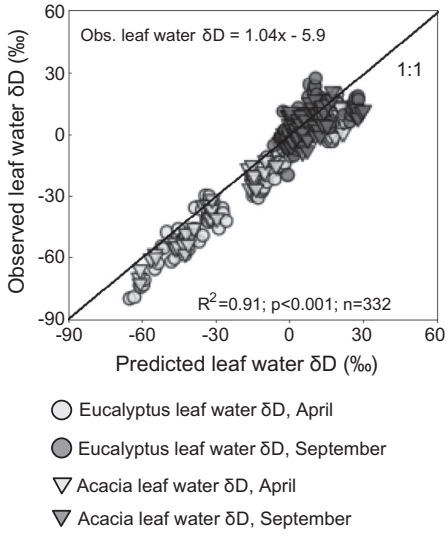

Fig. 4. Comparison between model-simulated and observed leaf water δD values for all *Eucalyptus* and *Acacia* species along the North Australia Transect for April and September 2010.

water δD values for each site along the climate gradient. The model simulation produced mean growing season leaf water δD values that increased from the tropical north to the arid south by 35‰ for *Eucalyptus* and 37‰ for *Acacia* (Fig. 5). The simulation shows that evaporative D-enrichment of leaf water can lead to substantial variation in leaf water δD values along environmental gradients such as the humidity gradient that we investigated here (Cernusak et al., 2005; Barbour, 2007; Kahmen et al., 2011a).

It is important to note, however, that our simulation of mean seasonal leaf water δD values is associated with uncertainties. This is because true growing season means of the four key model input variables (temperature, relative humidity, xylem water δD and atmospheric vapor δD) were only available for temperature, relative humidity and xylem water δD. In contrast, true growing season means of atmospheric vapor δD was not available as it was not possible to assess empirically seasonal means of this value for such a large gradient. We decided therefore to use the measured water vapor δD values from the September campaign as best estimates of the growing season mean water vapor δD values in our simulations. We are aware that the September values could deviate from the true growing season mean water vapor δD values and that this could possibly introduce errors to our simulations. For example, a 10‰ estimate error in atmospheric water vapor δD can cause a 3–5‰ deviation in simulated leaf water δD, depending on the relative humidity of a site. Despite these potential inaccuracies in our model simulation, we feel confident that the overall patterns in leaf water δD values that we simulated for the transect are correct. This is because our use of measured water vapor δD values in the simulations (in contrast to assumption-based estimates of these values) should keep uncertainties in water vapor δD values within reasonable limits. In addition, three of the four most critical model input variables, mean seasonal temperature, relative humidity, and xylem water δD were available for the simulations as true growing season means, yielding confidence that the simulated leaf water δD values along the transect are realistic.

Another potential source of error in our model simulations is the type of leaf water model used. We decided to use the simplest version, the basic Craig Gordon model for our simulations (Eq. (1)). This basic model typically overpredicts the evaporative D-enrichment of leaf water (Barbour et al., 2004; Cernusak et al., 2005; Kahmen et al., 2008). Along the climate gradient, the overprediction of leaf water δD values was, however, relatively small and

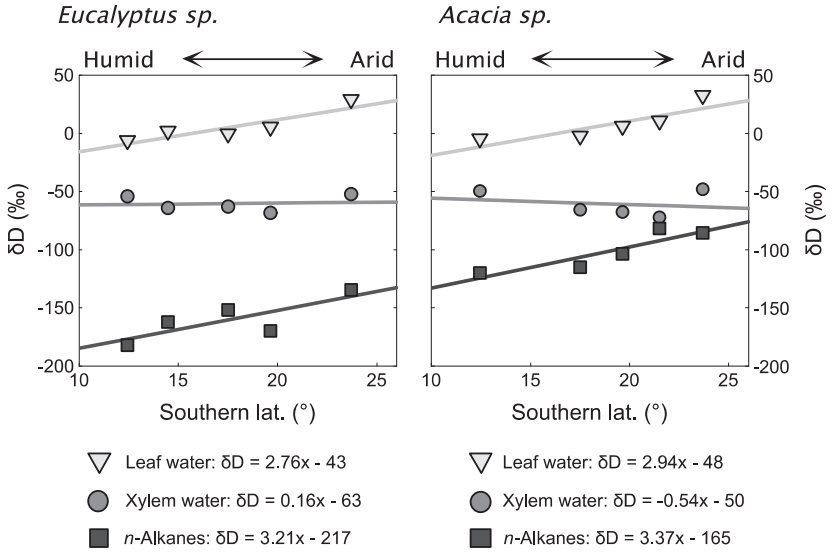

Fig. 5. The variation of leaf water, xylem water and leaf wax *n*-alkane δD values along the climate gradient. Leaf water values reflect modeled mean growing season (Oct–Nov–Dec) midday leaf water. Xylem water δD and leaf wax *n*-alkane δD values are empirical data reflecting the means of three replicate plants. Standard deviations for all δD values were below 11‰ and thus too small to be displayed in the figure.

Table 2
Concentration of individual leaf wax *n*-alkanes in the leaves of *Eucalyptus* (*Corymbia*) and *Acacia* species along the North Australian Transect in μg *n*-alkane per g leaf dry mass. Values are the mean of three replicate plants, sd = one standard deviation of the mean.

Species	S-lat. (°)	*n*-C_{25}		*n*-C_{27}		*n*-C_{29}		*n*-C_{31}		*n*-C_{33}	
		Mean (μg/g)	sd	Mean (μg/g)	sd	Mean (μg/g)	sd	Mean (μg/g)	sd	Mean (μg/g)	sd
Corymbia terminalis	23.70	2	<1	18	6	3	1	2	<1	–	–
Acacia kempiana	23.70	2	<1	9	2	12	1	620	164	611	126
Acacia kempiana	21.52	3	<1	16	4	52	11	1454	113	900	77
Eucalyptus pruinosa	19.65	8	6	49	32	11	6	<1	<1	–	–
Acacia cowleana	19.65	2	<1	7	1	74	4	987	18	309	19
Corymbia terminalis	17.50	9	4	59	29	8	6	3	4	1	1
Acacia colei	17.50	2	<1	7	2	43	13	498	142	118	42
Corymbia foelscheana	14.48	1	<1	8	1	4	<1	<1	<1	<1	<1
Eucalyptus miniata	12.44	<1	<1	5	1	42	10	2	2	–	–
Acacia auriculiformis	12.44	1	<1	3	1	198	88	15	7	1	2

Table 3
δD values of individual leaf wax *n*-alkanes and the concentration weighted average leaf wax *n*-alkane δD values (CWA) in the leaves of *Eucalyptus* (*Corymbia*) and *Acacia* species along the North Australian Transect. Values are the mean of three replicate plants, sd = one standard deviation of the mean.

Species	S-lat. (°)	*n*-C_{25}		*n*-C_{27}		*n*-C_{29}		*n*-C_{31}		*n*-C_{33}		CWA	
		Mean (‰)	sd	Mean (‰)	sd	Mean (‰)	sd	Mean (‰)	sd	Mean (‰)	sd	Mean (‰)	sd
Corymbia terminalis	23.70	−139	–	−137	5	−129	4	–	–	–	–	−135	4
Acacia kempiana	23.70	–	–	–	–	–	–	−87	6	−86	4	−86	5
Acacia kempiana	21.52	–	–	–	–	–	–	−81	2	−84	2	−82	2
Eucalyptus pruinosa	19.65	−168	1	−172	4	−163	5	–	–	–	–	−170	4
Acacia cowleana	19.65	–	–	–	–	–	–	−104	2	−103	2	−104	2
Corymbia terminalis	17.50	–	–	−151	2	–	–	–	–	–	–	−151	2
Acacia colei	17.50	–	–	–	–	–	–	−115	3	−116	–	−115	2
Corymbia foelscheana	14.48	−163	10	−164	11	−159	9	–	–	–	–	−163	10
Eucalyptus miniata	12.44	–	–	−178	6	−182	2	–	–	–	–	−182	3
Acacia auriculiformis	12.44	–	–	–	–	−119	8	−102	9	–	–	−118	8

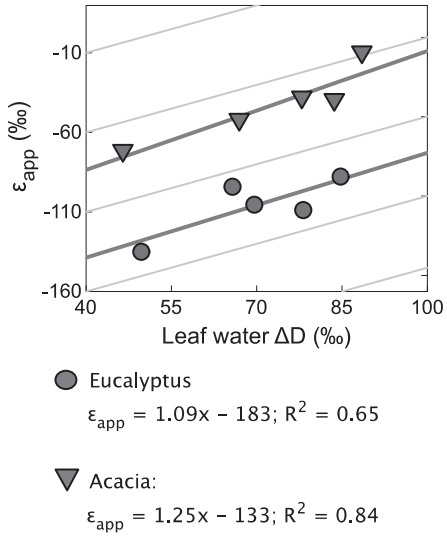

Fig. 6. The relationship between the apparent fractionation ε_{app} and leaf water evaporative enrichment above source water (ΔD) for both genera. The slope of the relationship indicates the fractional contribution of D-enriched leaf water to the pool of biosynthetic water. Grey lines indicate hypothetical 1:1 relationships between ΔD and ε_{app}, where leaf water would contribute 100% to the biosynthetic water pool.

averaged consistently 6‰. Given this relatively small overprediction, we decided to use the basic Craig–Gordon model for our simulations of mean seasonal leaf water δD values along the transect rather than using a more complex model, such as the Peclet-modified Craig–Gordon model (Barbour et al., 2004; Cernusak et al., 2005; Kahmen et al., 2011a). We decided against a more complex model, because it would require additional input variables (transpiration, effective pathlength). As discussed above, the true seasonal means of these variables are difficult to assess along a large gradient and their estimation could introduce error to the simulations that could be substantially larger than the relatively small overprediction of the basic Craig Gordon model that we observed here.

4.2. Relationship between leaf water and *n*-alkane δD values in natural ecosystems

The concentration-weighted leaf wax *n*-alkane δD values increased along the transect with more D-enriched values in more arid regions (Fig. 5). The increase in leaf wax *n*-alkane δD values followed the same direction as the increase in modeled mean seasonal leaf water δD values along the transect (Fig. 5). Since xylem water δD did not increase along the transect, this pattern suggests that D-enriched leaf

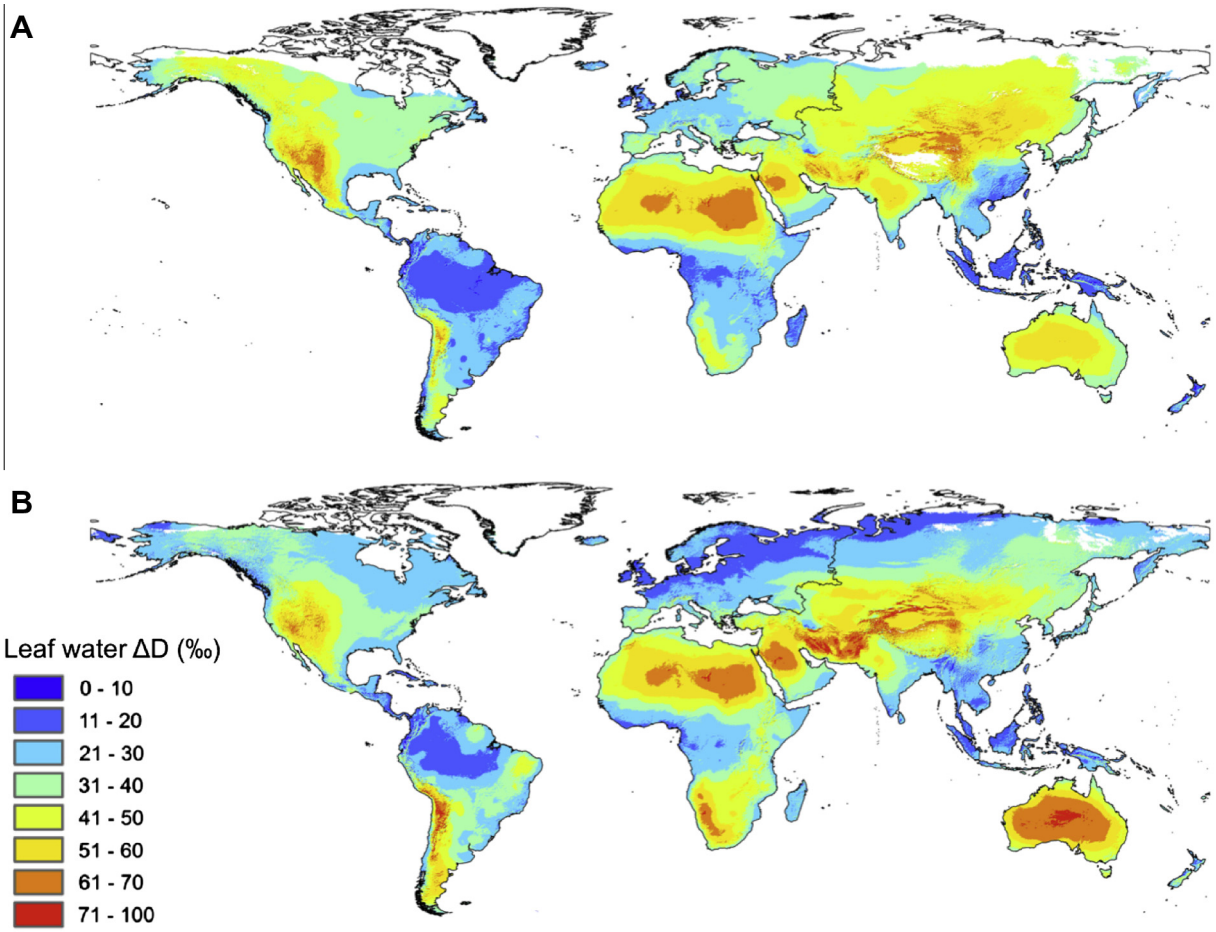

Fig. 7. Global variation of leaf water evaporative enrichment in deuterium (ΔD). Panel A shows ΔD values for the northern hemisphere spring (Apr–May–Jun), panel B ΔD values for the southern hemisphere spring (Oct–Nov–Dec). Grid cells with mean monthly temperatures less than 0 °C not included.

water is a critical driver of the leaf wax *n*-alkane δD values of both *Eucalyptus* and *Acacia* species along the transect (Fig. 5). This finding supports the results from our experimental investigations that we report in the companion paper and corroborates earlier studies that have anticipated leaf water evaporative D-enrichment to affect the δD values of leaf wax *n*-alkanes (Sachse et al., 2004, 2009, 2010; Smith and Freeman, 2006; Pedentchouk et al., 2008; Feakins and Sessions, 2010; Polissar and Freeman, 2010).

To quantify the effects of leaf water D-enrichment on leaf wax *n*-alkane δD values, we determined the degree of mean seasonal leaf water evaporative D-enrichment (ΔD, Eq. (4)) for each species along the climate gradient and related the resulting ΔD to their corresponding values of ε_{app} for the individual genera (Fig. 6). This allowed assessing the extent to which D-enriched leaf water contributes to the biosynthetic water pool and to quantify the influence on the δD values of leaf wax *n*-alkanes. We obtained slopes of 1.09 for *Eucalyptus* and 1.25 for *Acacia* from this analysis suggesting that D-enriched leaf water contributes 109% and 125% to the biosynthetic water pool in the two genera *Eucalyptus* and *Acacia*, respectively (Fig. 6). Our estimates of leaf water D-enrichment contributing 109% and 125% to the pool of biosynthetic water in *Eucalyptus* and *Acacia* exceed the maximum possible 100% that leaf water can theoretically contribute to the pool of biosynthetic water. These overestimations are possibly caused by the uncertainties in our model predicted mean seasonal leaf water δD values that we discussed above. It is, for example possible, that our use of September water vapor δD values (instead of true growing season means) led to underestimated leaf water evaporative D-enrichment along the gradient and thus overestimation of the contribution of leaf water to the biosynthetic water pool. In addition analytical error and biological variability could have resulted in a slight overestimation of the rate by which leaf wax *n*-alkane δD values increase along the gradient. Such sources of error are difficult to avoid in large-scale field studies such as the one we present here.

Although our analysis overpredicted the contribution of D-enriched leaf water on the biosynthetic water pool, the general effects of leaf water evaporative D-enrichment on leaf wax *n*-alkane δD values that we report here are in the same range as the effects of leaf water D-enrichment on leaf wax *n*-alkane δD values that we observed for dicotyledonous plants in our experimental investigations reported in the companion paper (Kahmen et al., 2013). The patterns we show here thus confirm our previous conclusion that the full extent of leaf water evaporative D-enrichment is recorded in the δD values of leaf wax *n*-alkanes for dicotyledonous plants. In addition to the δD values of precipitation, the D-enrichment of leaf water thus has an important influence on the δD of leaf wax *n*-alkanes, not only under controlled conditions as shown in our companion paper but also under natural conditions as demonstrated here.

Our study also shows that leaf water evaporative D-enrichment under natural conditions is indeed sufficient in magnitude to be a substantial driver of the variability in leaf wax *n*-alkane δD values. This finding is in contrast to conclusions from Hou et al. (2008), who questioned that leaf water D-enrichment is sufficient enough to have any effect on leaf wax δD values. Using the Craig–Gordon model, Hou et al. estimated bulk leaf water D-enrichment to be only marginal – even when following relative humidity changes of 40% – and concluded that such minor variations in leaf water D-enrichment should also have only a minor influence on the δD values of leaf wax lipids. However, McInerney et al. (2011) have pointed out that Hou et al. (2008) substantially overestimated dilution factors in their model of leaf water D-evaporative enrichment and thus underestimated evaporative D-enrichment of leaf water. The use of correct dilution factors would have resulted in similar sensitivities of leaf water δD values to evaporative D-enrichment that we describe here for the North Australia transect and therefore supports the conclusion that leaf water can become sufficiently enriched in D to be a possible source of variation for the δD values of leaf wax *n*-alkanes.

4.3. Variability of ε_{bio} within and among species

ε_{bio} has been reported to be rather insensitive to environmental variables when tested for freshwater green algae under controlled environmental conditions (Zhang and Sachs, 2007). ε_{bio} was also found to be constant for greenhouse grown C3 and C4 plants when temperature was varied (Zhou et al., 2011). We therefore assumed that ε_{bio} is a species-specific constant. However, to date only a few environmental parameters and their effects on ε_{bio} in higher plants have been tested. We therefore urge that future studies test our assumption of an environmentally insensitive ε_{bio}.

In our companion paper (Kahmen et al., 2013) we report substantial differences in ε_{bio} among different dicotyledonous plant species ranging from −145‰ to −209‰. Even more so, we found that ε_{bio} varied substantially between *Eucalyptus* and *Acacia* species along the North Australia transect (−183‰ and −133‰, respectively). As we suggest in Kahmen et al. (2013) differential contributions from distinct NADPH sources to hydrogen in leaf wax *n*-alkanes could also explain the differences in ε_{bio} between *Eucalyptus* and *Acacia* (Luo et al., 1991; Schmidt et al., 2003; Zhang et al., 2009). Additionally, the large differences in ε_{bio} of *Eucalyptus* and *Acacia* trees may be due to their very different leaf morphologies and anatomies. *Eucalyptus* species with an ε_{bio} of −183‰ and thus comparable to the range of values from the greenhouse grown dicotyledonous plants, have conventional dicotyledonous leaves. Many *Acacias*, particularly in Australia, have reduced or entirely absent leaves with prominent phyllodes. In a morphological sense phyllodes are secondary leaves developed from petioles and in contrast to normal dicotyledonous leaves have a parallel venation. These characteristic differences may explain why the estimated ε_{bio} of −133‰ for *Acacia* is significantly lower than for all other investigated plants in the field and in the greenhouse. Clearly, further research is needed to test and identify the drivers of the species-specific differences in ε_{bio} that we describe here and in the companion paper. Such investigations could help to understand mechanistically the substantial scatter among species that has been observed when leaf wax *n*-alkane δD values of dif-

ferent plant species have been assessed along environmental gradients (Chikaraishi and Naraoka, 2003; Sachse et al., 2006, 2012; Hou et al., 2007; Liu and Yang, 2008; Feakins and Sessions, 2010; Yang et al., 2011). In addition, it should be tested in future studies if the species-specific differences in ε_{bio} that we observed are randomly distributed among different plant species, or if these differences can be attributed to plants with particular environmental requirements. If ε_{bio} varies systematically among plants with different environmental requirements, environmentally-induced shifts in vegetation will also cause systematic changes in ε_{bio} and could as such affect the δD values of leaf wax *n*-alkanes.

4.4. Global variations in the effect of D-enriched leaf water on *n*-alkane δD values

Our observations along the climate gradient show that in addition to the δD of precipitation, D-enrichment of leaf water has a strong effect on the δD values of leaf wax *n*-alkanes. The magnitude of leaf water D-enrichment can, however, be substantially different in biomes that differ in their climate regimes. The magnitude of the leaf water effect on the δD values of leaf wax *n*-alkanes will therefore also differ among biomes that differ in their climate. To estimate differences in the magnitude by which leaf water D-enrichment influences the δD values of leaf wax *n*-alkanes in different biomes, we simulated global patterns of leaf water evaporative D-enrichment above source water (ΔD) using an isoscape model (West et al., 2008). The model simulation predicted mean growing season midday leaf water ΔD values for all parts of the globe, but with substantial spatial variability (Fig. 7). The predicted D-enrichment of leaf water was strongest in arid biomes (40–100‰), intermediate in temperate biomes (10–30‰) and least pronounced in tropical biomes (0–20‰). Hence we conclude that the influence of D-enriched leaf water on the δD values of leaf wax *n*-alkanes is particularly strong in arid and temperate environments and weaker in humid tropical environments. Based on these simulations, we predict that δD values of leaf wax *n*-alkanes from (wet) tropical environments will largely reflect δD values of precipitation, while the δD of leaf wax *n*-alkanes from arid or temperate environments will additionally reflect the plant physiological evapotranspiration processes that drive the D-enrichment of leaf water.

5. CONCLUSIONS

Both our climate chamber experiment that we report in the companion paper (Kahmen et al., 2013) and our observational study along the North Australian transect provide strong evidence for the influence of D-enriched leaf water on the δD values of leaf wax *n*-alkanes from angiosperm plants. Although we show that this effect is stronger for dicotyledonous plants than for monocotyledonous plants in our experiment (Kahmen et al., 2013), the results of both studies imply that the δD values of leaf wax *n*-alkanes of all plants are affected by evaporative D-enrichment of leaf water. This finding has important implications for the interpretation of leaf wax *n*-alkanes from sedimentary records, biomass samples or airborne dust, since it shows that hydrological information (i.e. δD of precipitation) *and* plant physiological information (i.e. the evapotranspiration processes that determine the D-enrichment of leaf water) are both recorded in the δD values of leaf wax *n*-alkanes.

Despite the combined hydrological and physiological information that is contained in the δD values of leaf wax *n*-alkanes, we suggest that δD values of leaf wax *n*-alkanes can be employed to obtain *general* hydrological information. This will be particularly true since precipitation δD values and leaf water D-enrichment both increase with increasing aridity and decrease with increasing humidity (Gat, 1996; Kahmen et al., 2008). The evaporative D-enrichment of leaf water tends therefore to amplify the hydrological signal derived from precipitation δD so that, for instance, variations in δD recorded in sedimentary leaf wax *n*-alkanes are expected to reflect qualitative changes in the general hydrological balance of a catchment area.

Quantitative inferences from leaf wax *n*-alkane δD values can, however, be achieved when additional environmental information is available to separate the precipitation- and leaf water-derived signals in leaf wax *n*-alkane δD values. We suggest that with the use of additional proxies (e.g. aquatic biomarker δD values that are not affected by transpiration (Sachse et al., 2004; Seki et al., 2011), carbon isotopes to distinguish C3 and C4 vegetation and pollen data to distinguish monocot and dicot vegetation) specific hydrological *and* physiological information, e.g. on changes in terrestrial evapotranspiration can be obtained from the δD values of leaf wax *n*-alkanes. The influence of D-enriched leaf water on the δD values of leaf wax *n*-alkanes that we show in our study should thus not be considered a confounding factor that obscures the hydrological signals recorded in the δD values of leaf wax *n*-alkane δD values. Rather, it should be interpreted as an additional important signal that can be assessed with accompanying environmental proxies and that makes leaf wax *n*-alkanes attractive ecohydrological proxies not only for paleohydrological research but also for ecosystem sciences.

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