Organic Geochemistry 62 (2013) 62–67

Contents lists available at ScienceDirect

Organic Geochemistry

journal homepage: www.elsevier.com/locate/orggeochem

Abundance and distribution of leaf wax *n*-alkanes in leaves of Acacia and Eucalyptus trees along a strong humidity gradient in northern Australia

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ARTICLE INFO

Article history:
Received 17 April 2013
Received in revised form 16 June 2013
Accepted 7 July 2013
Available online 15 July 2013

ABSTRACT

Environmental parameters such as rainfall, temperature and relative humidity can affect the composition of higher plant leaf wax. The abundance and distribution of leaf wax biomarkers, such as long chain *n*-alkanes, in sedimentary archives have therefore been proposed as proxies reflecting climate change. However, a robust palaeoclimatic interpretation requires a thorough understanding of how environmental changes affect leaf wax *n*-alkane distributions in living plants. We have analysed the concentration and chain length distribution of leaf wax *n*-alkanes in Acacia and Eucalyptus species along a 1500 km climatic gradient in northern Australia that ranges from subtropical to arid. We show that aridity affected the concentration and distribution of *n*-alkanes for plants in both genera. For both Acacia and Eucalyptus *n*-alkane concentration increased by a factor of ten to the dry centre of Australia, reflecting the purpose of the wax in preventing water loss from the leaf. Furthermore, Acacian-alkanes decreased in average chain length (ACL) towards the arid centre of Australia, whereas Eucalyptus ACL increased under arid conditions. Our observations demonstrate that *n*-alkane concentration and distribution in leaf wax are sensitive to hydroclimatic conditions. These parameters could therefore potentially be employed in palaeorecords to estimate past environmental change. However, our finding of a distinct response of *n*-alkane ACL values to hydrological changes in different taxa also implies that the often assumed increase in ACL under drier conditions is not a robust feature for all plant species and genera and as such additional information about the prevalent vegetation are required when ACL values are used as a palaeoclimate proxy.

1. Introduction

Leaves of higher terrestrial plants are coated with a protective wax layer, the cuticle, that restricts uncontrolled water loss from leaves (Riederer and Markstaedter, 1996). The layer contains long chain (> 23 carbons) *n*-alkanoic acids, alcohols and *n*-alkanes (Eglinton et al., 1962). These compounds can accumulate in marine and lacustrine sediments and have been suggested as biomarkers for the reconstruction of ancient ecosystems or change in climate (Eglinton and Hamilton, 1967; Sachse et al., 2012). Other potential sources, including algae have been identified (Grice et al., 1998, 2001; Peters et al., 2005), although in most sedimentary archives the vast majority of these compounds likely originate from higher plant leaf waxes. *n*-Alkanes in particular are a focus for such reconstructions, as they are relatively resistant to degradation and easy to extract from sediments and purify.

The *n*-alkane chain length distribution of plant leaf wax in modern plants is sensitive to environmental conditions (Smolenska and Kuiper, 1977; Bulder et al., 1989; Maffei et al., 1993). It has therefore been suggested that changes in *n*-alkane composition can be used for the reconstruction of environmental change. For instance, Sachse et al. (2006) observed that broadleaf tree leaves along a N–S European gradient contained longer chain *n*-alkanes in the warmer (and dryer) regions than the same species in colder (and wetter) regions. Several studies have interpreted the average chain length index (ACL) of *n*-alkanes and *n*-alkanoic acids from sediment cores as proxies for hydroclimatic variability (Hughen et al., 2004; Rommerskirchen et al., 2006; Schouten et al., 2007). However, there are few data for modern plants, which systematically characterize and quantify *n*-alkane concentration and distributions in terrestrial plants along larger spatial scales. The studies which have investigated ACL variability along climatic gradients have either compared *n*-alkane concentration and distributions across different species (Sachse et al., 2006), or have focused on temperature variability (Tipple and Pagani, 2013). Studies examining the variability

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0146-6380/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.orggeochem.2013.07.003

in *n*-alkane concentration and distributions along hydrological gradients are missing. As such, the interpretation of ACL as a qualitative hydroclimatic proxy is associated with large uncertainty.

Here we specifically evaluate the effects of varying hydroclimatic conditions on the distribution and ACL of leaf wax *n*-alkanes in the leaves of different Acacia and Eucalyptus species along a 1500 km rainfall and humidity gradient in the Northern Territory, Australia.

1.1. Sampling

In April 2010 we collected fresh leaf samples of Acacia and Eucalyptus/Corymbia species, which dominate the vegetation along the 1500 km humidity gradient in the Northern Territory, Australia (Barlow et al., 1981; Bowman and Connors, 1996; Hutley et al., 2011). Precipitation, temperature and relative humidity at the sampling sites were constantly monitored by the Bureau of Meteorology of the Australian Government. Weather stations are within a 5 km radius of the sampling sites (except Alice Springs – 12 km, Territory Grape Farm – 32 km, Katherine – 11 km, Adelaide River – 9 km; see also Kahmen et al., 2013). Climate along the gradient ranges from subtropical at Darwin at the Timor Sea coast to arid at Alice Springs in central Australia. The 30 yr average (1976–2005) of annual mean precipitation (*P*) at Darwin is 1705 mm and decreases towards Alice Springs to 278 mm (Fig. 1). The average annual mean relative humidity ranges from 31.6% in the south (Barrow Creek) to 62.3% in the north (Darwin). In contrast, annual mean temperature shows a limited range from 24.2 °C at Alice Springs to 29.4 °C at Katherine. The elevation rises moderately from 30 m above sea level (a.s.l.) at the lowermost sample site (Darwin in the north) to 566 m a.s.l. at Territory Grape Farm in the south.

Eucalyptus and Corymbia are closely related genera (Hill and Johnson, 1995). In order to simplify terminology here they are referred to collectively as Eucalyptus. Two different Eucalyptus species were sampled at Darwin, Adelaide River, Larrimah, Katherine, Elliot, Tennant Creek and Alice Springs, and at all other sites only one. In total, we collected 18 Eucalyptus samples from 11 species along the transect. We sampled one Acacia species at each site along the gradient except at Katherine and Adelaide River. In total, we collected 9 Acacia samples from 7 species along the transect. A detailed list is given in Table 1. For each species and location we collected samples from six individual trees; 10–50 leaves per replicate plant were harvested randomly from the crown to reflect an average tree value. Leaves for *n*-alkane analysis were stored in paper bags and dried for several days. For details of the sampling strategy see Kahmen et al. (2013).

An additional set of samples for leaf area determination was collected at only five sites (Darwin, Katherine, Elliot, Tennant Creek and Alice Springs). These samples were stored in ziplock plastic bags and kept cool until leaf area determination in the laboratory. Leaf area was measured using a LI 3100 leaf area meter (Licor, Lincoln, NE, USA) and leaves were dried at 80 °C for 48 h prior to dry weight determination of each sample.

2. Methods

2.1. Leaf wax extraction and quantification

Dry leaves were ground with a flint mill to a fine powder. In total, 81 samples were extracted and analysed – three out of the collected six replicate samples per species per sample site were extracted for lipid biomarkers and analysed separately. For extraction, we used between 0.50 and 0.75 g of ground plant material. Each sample was sonicated [15 min; dichloromethane(DCM)/MeOH, 9:1, 30 ml]. The mixture was filtered and excess solvent evaporated. To the dry total lipid extract (TLE) in 1.5 ml *n*-hexane

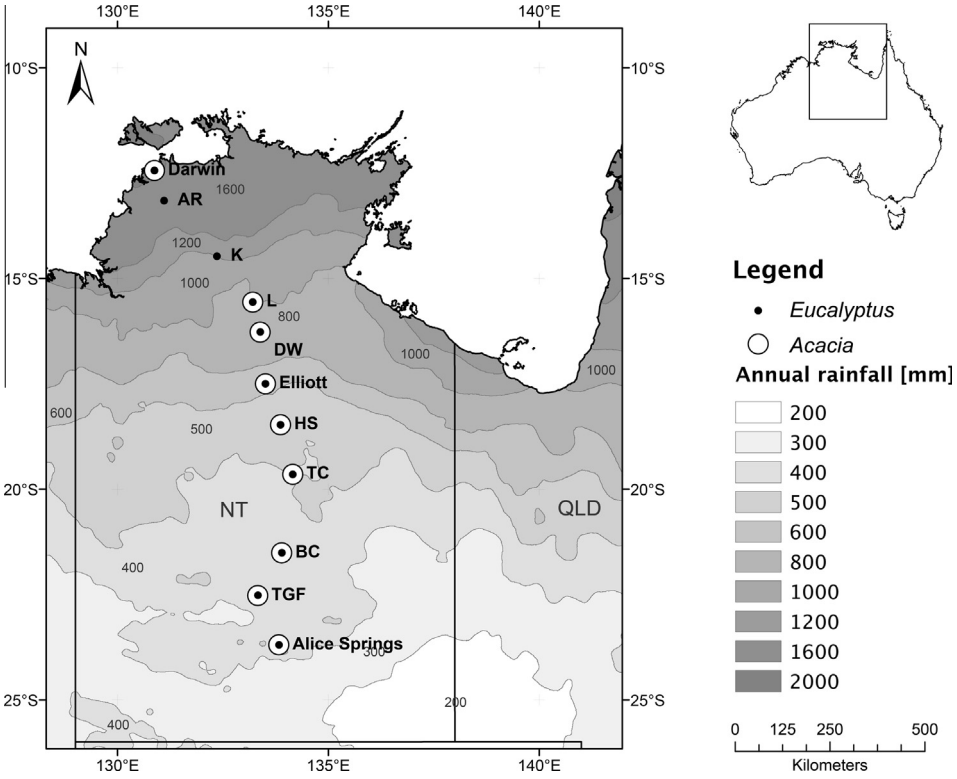

Fig. 1. Location of sampling sites and mean annual precipitation in the Australian Northern Territory (NT). Circle size indicates sampled tree genus at each site (AR, Adelaide River; K, Katherine; L, Larrimah; DW, Daly Waters; HS, Helen Springs; TC, Tennant Creek; BC, Barrow Creek; TGF, Territory Grape Farm).

Table 1
Species, site location and leaf wax *n*-alkane parameters.

Species	Site name	Latitude	ACL	con_w[a] (μg g^{-1})	con_w 1 rel. stdev. (%)	con_a[b] (μg cm^{-2})	con_a 1 rel. stdev. (%)	CPI
Acacia								
Acacia auriculiformis	Darwin	12.44	29.11	238	44	26.4	18	10.57
Acacia sp.	Larrimah	15.56	31.47	145	10			8.22
Acacia shirleyi	Daly Waters	16.27	29.96	210	23			6.69
Acacia colei	Elliott	17.50	31.15	740	31	181.1	6	9.09
Acacia colei	Helen Springs	18.47	31.28	817	12			11.29
Acacia cowleana	Tennant Creek	19.65	31.31	1486	2	380.2	9	12.92
Acacia kempiana	Barrow Creek	21.52	31.58	2752	6			7.43
Acacia aneura	Territory Grape Farm	22.53	32.10	2818	69			11.80
Acacia kempiana	Alice Springs	23.70	31.88	1340	21	285.2	21	14.34
Eucalyptus								
Eucalyptus tetrodonta	Darwin	12.44	28.99	58	25	10.3	17	14.25
Eucalyptus miniata	Darwin	12.44	28.72	56	14	9.1	6	7.88
Corymbia confertiflora	Adelaide River	13.16	27.85	10	34			4.02
Eucalyptus tectifica	Adelaide River	13.16	28.99	16	34			3.32
Corymbia foelscheana	Katherine	14.48	27.35	17	7	3.8	2	3.42
Eucalyptus tectifica	Katherine	14.48	28.28	38	66	8.6	2	4.18
Eucalyptus tetrodonta	Larrimah	15.56	28.31	90	72			7.86
Corymbia dichromophloia	Larrimah	15.56	27.57	22	10			2.36
Corymbia polycarpa	Daly Waters	16.27	27.13	28	5			2.93
Eucalyptus pruinosa	Elliott	17.50	27.05	155	8	39.9	5	3.18
Corymbia terminalis	Elliott	17.50	26.83	110	55	28.9	6	2.86
Eucalyptus pruinosa	Helen Springs	18.47	27.70	66	33			3.63
Eucalyptus pruinosa	Tennant Creek	19.65	26.87	89	67	20.8	3	3.51
Eucalyptus leucophloia	Tennant Creek	19.65	28.22	117	34	23.5	17	5.07
Corymbia terminalis	Barrow Creek	21.52	27.38	30	14			4.15
Corymbia terminalis	Territory Grape Farm	22.53	27.22	18	46			3.53
Corymbia aparrerinja	Alice Springs	23.70	27.01	265	32	66.8	20	5.89
Corymbia terminalis	Alice Springs	23.70	27.14	34	25			2.78

[a] Relative *n*-alkane concentration per leaf wt, (with stdev. of triplicate samples).
[b] Relative *n*-alkane concentration per leaf area (with stdev. of triplicate samples).

was added 20 μg 5α-androstane as internal standard. The TLE was fractionated using a 10 ml solid phase columns filled with ca. 1.5 g silica gel (0.040 mm to 0.063 mm mesh size). After the column was cleaned with *n*-hexane, DCM and acetone, the TLE in *n*-hexane was loaded onto the column and *n*-alkanes eluted with 8 ml *n*-hexane. The eluent was evaporated to dryness, re-dissolved in 1 ml *n*-hexane and transferred to a 2 ml vial. Two additional fractions, ketones (1:1 *n*-hexane/DCM) and polar compounds (1:1 DCM/MeOH), were eluted and archived (data not shown). The *n*-alkanes were analyzed using an Agilent 5975C Series mass selective detector (MSD) system equipped with an Agilent 7890A gas chromatography (GC) instrument and flame ionization detection (FID). An aliquot (1 μl) of sample was injected (Agilent 7683B Series) as follows: 30 m Agilent J&W HP-5ms column, 70–320 °C (held 15 min) at 12 °C/min. Leaf wax *n*-alkanes were quantified relative to the internal standard (FID).

2.2. Climate data along gradient

We used the 30 yr mean annual temperature (T_{ann}), relative humidity (RH_{ann}), and the leaf-to-air vapour pressure difference (VPD_{ann}), as well as mean annual precipitation amount at the appropriate sample site. Additionally, we compared the leaf wax *n*-alkane data with annual potential evapotranspiration (PET_{ann}; Morton, 1983) and aridity index (AI), defined as the ratio of annual precipitation and PET_{ann} (Budyko, 1974). All climate data were obtained from the Commonwealth of Australia 2011, Bureau of Meteorology.

2.3. Data evaluation

The *n*-alkane concentration was normalized to dry leaf wt. (con_w) and leaf area (con_a). In order to characterize total leaf wax distribution per species and location, we calculated the weighted average chain length (ACL) of *n*-alkanes from:

$$\mathrm{ACL} = \sum nx\,con_w(n) / \sum con_w(n) \quad (1)$$

where *n* is the number of carbon atoms in an *n*-alkane and $con_w(n)$ is the appropriate concentration per leaf dry wt.

For data evaluation and calculation of con_w,con_a and ACL we used mean values of the three replicate samples and report their standard deviation in Table 1.

3. Results and discussion

3.1. n-Alkane concentration per leaf weight (con_w)

Both genera contained abundant long-chain *n*-alkanes with 23–33 carbons. Typical for higher plants, they showed a strong odd/even predominance (CPI, from 2.4 to 14.3). The dominant *n*-alkane in Eucalyptus was C_{27}, while C_{31} dominated in Acacia.

In general, the concentration of *n*-alkanes per g dry wt. in Acacia leaves was one order of magnitude greater than in Eucalyptus leaves. The total *n*-alkane concentration per leaf dry wt. in Acacia samples ranged from 145 μg g^{-1} at Larrimah to 2818 μg g^{-1} to Territory Grape Farm (Table 1). Total *n*-alkane concentration in Eucalyptus leaves varied (Table 1) between 10 μg g^{-1} at Adelaide River (*Corymbia confertiflora*) and 265 μg g^{-1} at Alice Springs (*Corymbia aparrerinja*). The concentrations on a dry leaf wt. basis were within the range reported for other dicotyledonous plants [e.g. Eglinton et al. (1962) for *Crassulaceae* leaves (200–27.5 × 10^3 μg g^{-1}), Maffei (1996) for *Rosmarinus officinalis* leaves (880–2360 μg g^{-1}), Kahmen et al. (2011) for *Populus trichocarpa* leaves (2100–3400 μg g^{-1}) and Sachse et al. (2010) for *Hordeum vulgare* leaves (30–50 μg g^{-1})].

Leaf wax *n*-alkane concentration of both genera increased towards the arid southern end of the transect. The log *n*-alkane concentration per g leaf dry wt. (con_w) of *Acacia* samples showed significant correlations with PET_{ann} (r −0.83, p = 0.006) and RH_{ann} (r −0.80, p = 0.012). Con_w of Eucalyptus leaves also increased

towards the southern end of the transect, but the trend did not correlate significantly with any given climate parameter.

3.2. n-Alkane concentration per leaf area (con_a)

The *n*-alkane concentration per leaf area (con_a) generally increased with increasing aridity for both genera. For Acacia leaves it ranged (Table 1) from 26.4 μg cm^{-2} (*Acacia auriculiformis*) at Darwin to 380.2 μg cm^{-2} at Tennant Creek (*Acacia cowleana*). Eucalyptus samples again showed much lower concentration per leaf area than Acacia, varying from 3.8 μg cm^{-2} at Katherine (*Corymbia foelscheana*) to 66.8 at Alice Springs (*C. aparrerinja*). The con_a values for Acacia and Eucalyptus were of the same order of magnitude as reported for other dicotyledonous plants (Herbin and Robins, 1969; Baker, 1974).

Con_a of Acacia leaves correlated significantly with RH_{ann} (r −1.00, $p = 0.001$), annual precipitation (r −0.98, $p = 0.024$) and aridity (r −0.97, $p = 0.026$). Con_a of Eucalyptus leaves correlated with PET_{ann} (r −0.85, $p = 0.004$) annual precipitation (r −0.74, $p = 0.021$) and aridity (r −0.74, $p = 0.021$). Con_a of Acacia and Eucalyptus did not correlate with mean annual temperature, probably due to the small range of T_{ann} between 24.17 °C (Alice Springs) and 29.36 °C (Katherine). In summary, con_w was less strongly correlated with hydrological parameters than con_a.

Correlation for con_a contained fewer data points than for con_w because leaf area was assessed only at five sites. If the number of samples used for con_w correlation (between 8 and 18, depending on sample # per genus and RH data availability) was reduced to the number of samples used for con_a correlation (4–9), environmental variables could explain greater variability in con_w than con_a but the correlation between con_w and environmental variables was still of lower significance than for between con_a and climate variables. These observations reflect the situation that *n*-alkanes are synthesized on the leaf surface, where they function as a protective coating to prevent water loss due to uncontrolled transpiration through the epidermal cells.

3.3. n-Alkane distribution and ACL

ACL for Eucalyptus *n*-alkanes varied between 26.83 and 29.34 and decreased from the moist northern sites towards the arid south (Table 1, Fig. 2). The opposite trend was observed for Acacia. ACL of Acacia *n*-alkanes varied between 29.11 and 32.10 and

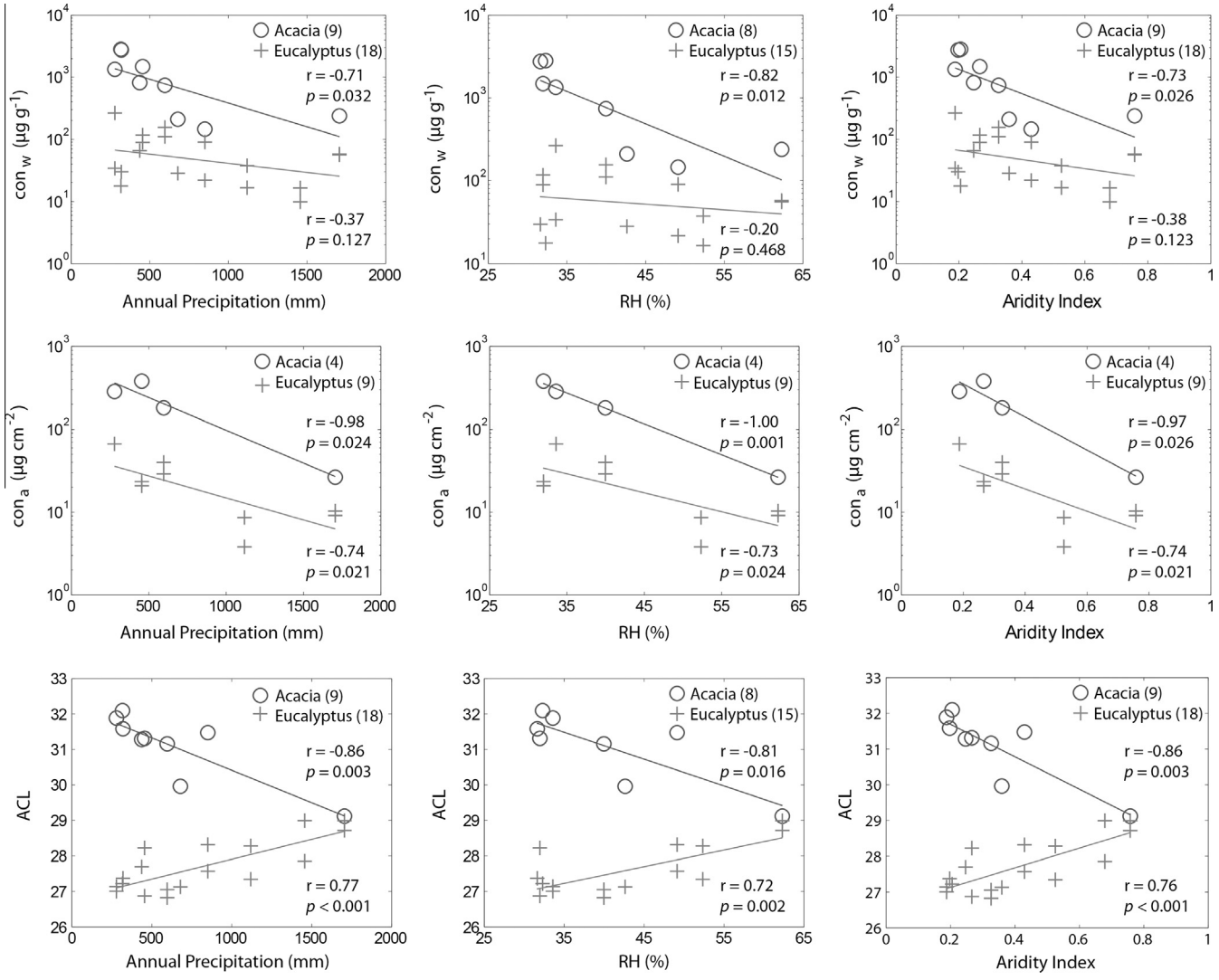

Fig. 2. *n*-Alkane concentration per leaf surface area (con_a, log scale) and per leaf wt. (con_w, log scale) and ACL vs. mean annual precipitation rate, mean annual relative humidity (RH) and aridity index. The strongest correlation was between con_a and precipitation, as well as RH. The number of samples per genus (in brackets) varied due to limited con_a and RH data sets.

increased from Darwin towards the south (Fig. 1). The highest value for Acacia (32.10) was at Territory Grape Farm (*Acacia aneura*).

ACL of both genera correlated significantly with variables reflecting the sample site hydrological conditions (RH_{ann}, annual precipitation, PET_{ann} and aridity), albeit in opposite directions (Table 2, Fig. 2). This indicates that water availability and evapotranspiration were the major drivers of leaf wax *n*-alkane chain length distribution along the gradient. We observed no relationship between ACL and T_{ann}, which has been identified as a factor affecting ACL (Tipple and Pagani, 2013). However, our dataset covers only a relatively small range of T_{ann} of 5 °C (see also Section 3.2) compared with the > 12 °C range from Tipple and Pagani (2013).

The reason for opposite trends in *n*-alkane ACL vs. hydroclimate relationship for both genera is not known. It is conceivable that the known evolutionary differences between Acacia and Eucalyptus led to the patterns. In contrast to Eucalyptus, Acacia species have flattened petioles (phyllodes) rather than true leaves with the lamina absent. Comparison of the hydrogen stable isotopic composition (δD value) of Acacia and Eucalyptus leaf wax *n*-alkanes by Kahmen et al. (2013) also suggests that biochemical differences exist among Acacia phyllodes and Eucalyptus leaves. It is possible that these differences led to both isotopic and compositional differences, although our dataset did not allow a more thorough evaluation.

Interestingly, studies addressing leaf wax *n*-alkane ACL as a climatic proxy found that T_{ann} was a major determinant of ACL variation. Sachse et al. (2006) found an increase in leaf wax *n*-alkane ACL of Betula species (sampled from northern to southern Europe) with increasing T_{ann}. However, in southern Europe, climatic conditions become dryer with increasing temperature and these two variables were not evaluated separately. Tipple and Pagani (2013) also found a relationship between T_{ann} and ACL of leaf wax from angiosperms (*Platanus occidentalis* and *Acer rubrum*), gymnosperms (*Juniperus virginiana* and *Pinus strobus*/*taeda*) and soils along a North American transect. In addition, they reported a negative correlation between ACL of pine tree leaf wax *n*-alkanes and T_{ann}. The reported range of ACL values along the whole transect covering a 12 °C temperature difference is for all species below one ACL unit, indicating a relatively low sensitivity with regard to T_{ann}. In comparison, the ACL variability along our hydrological gradient was up to three and two ACL units for Acacia and Eucalyptus, respectively. Together with previous studies, our data suggest that temperature and aridity can both affect leaf wax composition and that changes in hydrological conditions seem to have a stronger effect on ACL than mean annual temperature.

3.4. Consequences for applying leaf wax *n*-alkane distributional changes in sediment cores as palaeoenvironmental proxies

Our study shows that leaf wax *n*-alkane composition is affected by environmental variables. As a consequence, changes in leaf wax distributions in sediments could potentially be used as a proxy for palaeoclimate change. The opposing trends for the relationship of ACL with RH and AI relationships in Acacia and Eucalyptus leaves (Fig. 2) along the gradient imply, however, that care has to be taken when ACL is to be used as a climate proxy. Our results demonstrate a correspondence with previous studies (Sachse et al., 2006; Tipple and Pagani, 2013) in that changes in vegetation cover could significantly affect sedimentary ACL records irrespective of climate. The often assumed increase in ACL under drier conditions (Bulder et al., 1989; Sachse et al., 2006; Eglinton and Eglinton, 2008) is therefore not a robust feature for all plant species and genera. We argue that the ACL index for sedimentary leaf wax *n*-alkanes can only be applied as a palaeoclimatic proxy under the assumption of a stable vegetation cover within the catchment and no additional external leaf wax sources and that it will be difficult to interpret ACL as palaeoclimate proxy without accompanying additional proxy data, such as pollen analysis or leaf wax δD values, reflecting leaf water evapotranspiration.

Despite the increase here in *n*-alkane concentration under dry conditions, the use of sedimentary leaf wax *n*-alkane concentration as a palaeoclimatic indicator is also associated with uncertainty. Concentration differences among different plant species, change in vegetation cover and changing sedimentation rate, as well as changing erosion processes in the catchment area, also affect sedimentary *n*-alkane concentration and will likely override the plant

Table 2
Pearson product-moment correlation coefficient (*r*) and *p* values for different *n*-alkane parameters and climate variables (*n*, number of data points per correlation).

ACL	T_{ann}[a]	RH_{ann}[b]	VPD_{ann}[c]	Annual precipitation	PET_{ann}[d]	Aridity[e]
Acacia	*n* = 8	*n* = 8	*n* = 8	*n* = 9	*n* = 9	*n* = 9
r	−0.62	−0.81	0.55	−0.86	−0.85	−0.86
p	0.101	0.016	0.161	0.003	0.004	0.003
Eucalyptus	*n* = 15	*n* = 15	*n* = 15	*n* = 18	*n* = 18	*n* = 18
r	0.34	0.72	−0.69	0.77	0.70	0.76
p	0.215	0.002	0.004	<0.001	0.001	<0.001
n-Alkanes per wt. (con_w)						
Acacia	*n* = 8	*n* = 8	*n* = 8	*n* = 9	*n* = 9	*n* = 9
r	−0.76	−0.82	0.45	−0.71	−0.83	−0.73
p	0.028	0.012	0.267	0.032	0.006	0.026
Eucalyptus	*n* = 15	*n* = 15	*n* = 15	*n* = 18	*n* = 18	*n* = 18
r	−0.09	−0.20	0.21	−0.37	−0.36	−0.38
p	0.751	0.468	0.446	0.127	0.148	0.123
n-Alkanes per area (con_a)						
Acacia	*n* = 4	*n* = 4	*n* = 4	*n* = 4	*n* = 4	*n* = 4
r	−0.48	−1.00	0.84	−0.98	−0.92	−0.97
p	0.521	0.001	0.155	0.024	0.082	0.026
Eucalyptus	*n* = 9	*n* = 9	*n* = 9	*n* = 9	*n* = 9	*n* = 9
r	−0.61	−0.73	0.53	−0.74	−0.85	−0.74
p	0.079	0.024	0.138	0.021	0.004	0.021

[a] Annual mean temperature.
[b] Annual mean relative humidity.
[c] Annual mean vapour pressure difference.
[d] Annual potential evapotranspiration rate.
[e] Precipitation/potential evapotranspiration).

derived signal. Therefore, only if all these factors in a given sedimentary basin are known or can be assumed to have been constant over time, can an increase in leaf wax *n*-alkane concentration be related to higher leaf wax production rate as a result of more arid climatic conditions.

4. Conclusions

We observed a strong dependence of leaf wax *n*-alkane concentration and distribution (ACL) in different Acacia and Eucalyptus species on hydroclimatic conditions, which suggests that hydrology is an important factor controlling the leaf wax production and composition in these genera. Under dry conditions, both genera showed substantially higher concentration of *n*-alkanes. Leaf wax *n*-alkane concentration per leaf area correlated more strongly with hydrological variables than concentration per leaf wt., reflecting the purpose of leaf wax to restrict water loss from the leaf surface. Since ACL showed an increase under dry conditions for Acacia but a decrease for Eucalyptus, we urge caution in employing ACL as a simple palaeoenvironmental indicator in the absence of other proxy data. Detailed information on vegetation structure is needed when ACL values are to be used as a palaeoclimate proxy.

Acknowledgements

D.S. was supported by an Emmy-Noether Research grant from the German Science Foundation (DFG SA-1889/1-1). A.K. was supported by an ERC Starting Grant (279518 COSIWAX). L.A.C. was supported by a Discovery Grant (DP0771427) and a Future Fellowship (FT100100329) from the Australian Research Council, and by a Project Grant from Charles Darwin University. S.K.A. was supported by a Linkage Grant (LP100100073) from the Australian Research Council. We thank P. Atahan and an anonymous reviewer for helpful comments.

Associate Editor—**K. Grice**

References

Baker, E.A., 1974. The influence of environment on leaf wax development in *Brassica oleracea* var. *gemmifera*. New Phytologist 73, 955–966.

Barlow, B.A., Clifford, H.T., George, A.S., Kanis, A., McCusker, A., 1981. Flora of Australia. Volume 1. Introduction. AGPS / CSIRO. Available from: http://www.environment.gov.au/biodiversity/abrs/publications/flora-of-australia/vol01-1.html.

Bowman, D., Connors, G., 1996. Does low temperature cause the dominance of Acacia on the Central Australian Mountains? Journal of Biogeography 23, 245–256.

Budyko, M.I., 1974. Climate and Life, Hydrology and Earth System Sciences Discussions. Academic Press, New York.

Bulder, H., Leij, W., Speek, E., Van Hasselt, P., Kuiper, P., 1989. Interactions of drought and low temperature stress on lipid and fatty acid composition of cucumber genotypes differing in growth response at suboptimal temperature. Physiologia Plantarum 75, 362–368.

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

Eglinton, G., Hamilton, R.J., 1967. Leaf epicuticular waxes. Science 156, 1322–1335.

Eglinton, G., Gonzalez, A., Hamilton, R., Raphael, R.J., 1962. Hydrocarbon constituents of the wax coatings of plant leaves: a taxonomic survey. Phytochemistry 1, 89–102.

Grice, K., Schouten, S., Nissenbaum, A., Charrach, J., Sinninghe Damste, J.S., 1998. A remarkable paradox: sulfurised freshwater algal (*Botryococcus braunii*) lipids in an ancient hypersaline euxinic ecosystem. Organic Geochemistry 28, 195–216.

Grice, K., Audino, M., Boreham, C.J., Alexander, R., Kagi, R.I., 2001. Distributions and stable carbon isotopic compositions of biomarkers in torbanites from different palaeogeographical locations. Organic Geochemistry 32, 1195–1210.

Herbin, G.A., Robins, P.A., 1969. Patterns of variation and development in leaf wax alkanes. Phytochemistry 8, 1985–1998.

Hill, K.D., Johnson, L.A.S., 1995. Systematic studies in the eucalypts 7. A revision of the bloodwoods, genus Corymbia (Myrtaceae). Telopea 6, 185–504.

Hughen, K., Eglinton, T.I., Xu, L., Makou, M., 2004. Abrupt tropical vegetation response to rapid climate changes. Science 304, 1955–1959.

Hutley, L.B., Beringer, J., Isaac, P.R., Hacker, J.M., Cernusak, L.A., 2011. A subcontinental scale living laboratory: spatial patterns of savanna vegetation over a rainfall gradient in northern Australia. Agricultural and Forest Meteorology 151, 1417–1428.

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

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

Maffei, M., 1996. Chemotaxonomic significance of leaf wax alkanes in the Gramineae. Biochemical Systematics and Ecology 24, 53–64.

Maffei, M., Mucciarelli, M., Scannerini, S., 1993. Environmental factors affecting the lipid metabolism in *Rosmarinus officinalis* L.. Biochemical Systematics and Ecology 21, 765–784.

Morton, F.I., 1983. Operational estimates of areal evapotranspiration and their significance to the science and practice of hydrology. Journal of Hydrology 66, 1–76.

Peters, K.E., Walters, C.C., Moldowan, J.M., 2005. The Biomarker Guide: Biomarkers and Isotopes in the Environment and Human History, vol. 1. Cambridge University Press, Cambridge.

Riederer, M., Markstaedter, C., 1996. Plant cuticles: an integrated functional approach. In: Cuticular Waxes: a Critical Assessment of Current Knowledge. BIOS Scientific Publishers, Oxford, pp. 189–200.

Rommerskirchen, F., Eglinton, G., Dupont, L., Rullkötter, J., 2006. Glacial/interglacial changes in southern Africa: compound-specific $\delta^{13}C$ land plant biomarker and pollen records from southeast Atlantic continental margin sediments. Geochemistry, Geophysics, Geosystems 7, Q08010. http://dx.doi.org/10.1029/2005GC001223.

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

Sachse, D., Gleixner, G., Wilkes, H., Kahmen, A., 2010. Leaf wax *n*-alkane δD values of field-grown barley reflect leaf water δD values at the time of leaf formation. Geochimica et Cosmochimica Acta 74, 6741–6750.

Sachse, D., Billault, I., Bowen, G.J., Chikaraishi, Y., Dawson, T.E., Feakins, S.J., Freeman, K.H., Magill, C.R., McInerney, F.A., Van Der Meer, M.T.J., Polissar, P., Schmidt, H., Sessions, A.L., 2012. Molecular paleohydrology: interpreting the hydrogen-isotopic composition of lipid biomarkers from photosynthesizing organisms. Annual Review of Earth and Planetary Sciences, 221–249.

Schouten, S., Woltering, M., Rijpstra, W.I.C., Sluijs, A., Brinkhuis, H., Sinninghe Damsté, J.S., 2007. The Paleocene–Eocene carbon isotope excursion in higher plant organic matter: differential fractionation of angiosperms and conifers in the Arctic. Earth and Planetary Science Letters 258, 581–592.

Smolenska, G., Kuiper, P.J.C., 1977. Effect of low temperature upon lipid and fatty acid composition of roots and leaves of winter rape plants. Physiologia Plantarum 41, 29–35.

Tipple, B.J., Pagani, M., 2013. Environmental control on eastern broadleaf forest species' leaf wax distributions and D/H ratios. Geochimica et Cosmochimica Acta 111, 64–77.