Title: Carbon isotope discrimination in leaves of the common paperbark tree, *Melaleuca quinquenervia,* as a tool for quantifying past tropical and subtropical rainfall

Running head: *Melaleuca quinquenervia* leaf carbon isotopes

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Abstract

Quantitative reconstructions of terrestrial climate are highly sought after but rare, particularly in Australia. Carbon isotope discrimination in plant leaves (Δ_{leaf}) is an established indicator of past hydroclimate because the fractionation of carbon isotopes during photosynthesis is strongly influenced by water stress. Leaves of the evergreen tree *Melaleuca quinquenervia* have been recovered from the sediments of some perched lakes on North Stradbroke and Fraser Islands, south-east Queensland, eastern Australia. Here, we examine the potential for using *M. quinquenervia* Δ_{leaf} as a tracer of past rainfall by analysing carbon isotope ratios ($\delta^{13}C$) of modern leaves. We firstly assess Δ_{leaf} variation at the leaf and stand scale and find no systematic pattern within leaves or between leaves due to their position on the tree. We then examine the relationships between climate and Δ_{leaf} for an 11 year timeseries of leaves collected in a litter tray. *M. quinquenervia* retains its leaves for 1-4 years; thus cumulative average climate data are used. There is a significant relationship between annual mean Δ_{leaf} and mean annual rainfall of the hydrological year for 1-4 years (i.e. 365-1460 days) prior to leaf fall (r^2=0.64, p=0.003, n=11). This relationship is marginally improved by accounting for the effect of pCO_2 on discrimination (r^2=0.67, p=0.002, n=11). The correlation between rainfall and Δ_{leaf}, and the natural distribution of *Melaleuca quinquenervia* around wetlands of eastern Australia, Papua New Guinea and New Caledonia offers significant potential to infer past rainfall on a wide range of spatial and temporal scales.

Introduction

Quantitative reconstructions of past climate parameters, such as temperature and precipitation, are considerably more useful than dimensionless, relative assessments of climate trends such as “wetter” or “drier”. Quantitative inferences enable direct comparison of the rate and magnitude of climate change and variability between different locations and time periods (e.g. Shakun & Carlson, 2010) and they can be used to explore fundamental questions in environmental and Earth science, such as the relationship between global atmospheric carbon dioxide concentration ($p\mathrm{CO}_2$) and temperature (Rohling *et al.*, 2012, Shakun *et al.*, 2012) and the sensitivity of rainfall to temperature change (Li et al. 2013). Furthermore, numerical climate estimates can be compared with climate models to assess their veracity (Phipps *et al.*, 2013) and used as inputs into hydrological (Henley *et al.*, 2011) and/or ecological (Prowse *et al.*, 2013, Verschuren *et al.*, 2000) models. Importantly, quantitative reconstructions can be calibrated with (Emile-Geay *et al.*, 2013b), and tested against (Verschuren *et al.*, 2000), instrumental records, thereby facilitating an estimate of the precision and accuracy of proxy-climate relationships.

Numerical estimates of prehistoric climate change from terrestrial archives are limited in time and space. Many records span less than 1000 years or are spatially restricted (e.g. ice core or coral records). The Australian continent, in particular, has a very limited number of quantitative palaeoclimate reconstructions extending more than 1000 years. The majority of Australian proxy rainfall records, such as those derived from corals and tree rings, are relatively short (< 500 years, e.g. Lough, 2007, O'Donnell *et al.*, 2015), and even though individual records can be merged to lengthen the temporal coverage, this process reduces the proxy’s ability to detect low frequency variability (Ault *et al.*, 2013, Emile-Geay *et al.*, 2013a). As a result of the limited number of archives, key quantitative reconstructions of Australian climate rely on archives that are distant from the Australian mainland (Neukom & Gergis, 2012). These reconstructions are, therefore, based on teleconnections that may not be stable through time (Gallant *et al.*, 2013). Quantitative climate estimates >1000 years in length include temperature reconstructions from pollen (Fletcher & Thomas, 2010, Lloyd & Kershaw, 1997) and branched glycerol dialkyl glycerol tetraethers (Woltering *et al.*, 2014). Although some (pollen-based) rainfall reconstructions exist (Kershaw *et al.*, 2004a, Kershaw & Nix, 1988, Kershaw *et al.*, 2004b), the majority of Australian hydrological reconstructions consist of non-quantitative lake water balance and speleothem oxygen isotope records (e.g. Barr *et al.*, 2014, Denniston *et al.*, 2013, Gouramanis *et al.*, 2013).

Carbon isotope discrimination recorded in leaf tissue (Δ_{leaf}) reflects the degree of moisture stress experienced by plants, along with factors including photosynthetic rate (Farquhar *et al.*, 1989). Importantly, Δ_{leaf} can be quantitatively related to variables driving moisture stress including vapour pressure deficit (Brett *et al.*, 2014, Turney *et al.*, 1999) and rainfall (Diefendorf *et al.*, 2010, Kohn, 2010). The relationship between leaf carbon isotope ratios ($\delta^{13}C$) and rainfall has been predominantly used to infer rainfall in pre-Quaternary settings since lengthy (i.e. > 1000 year) preservation of leaves in late Quaternary deposits is rare (Birks, 2001, Spicer, 1989). However, the potential of this proxy was demonstrated by Liu *et al.* (2011) who used $\delta^{13}C$ in plant remains from a mixture of species to infer relative changes in rainfall during the Little Ice Age in north-western China.

We have discovered a number of perched acid lakes on North Stradbroke and Fraser Islands, south-east Queensland, Australia, that preserve leaves of the tree species *Meleleuca quinquenervia* **(**Cav.) S.T. Blake (the broad-leaved paperbark;

family Myrtaceae) in sediments deposited throughout much of the Holocene. In this study we assess the nature and strength of the rainfall-Δ_{leaf} relationship in *M. quinquenervia* to evaluate its potential as a rainfall proxy. Firstly, we characterize within-leaf and within-stand Δ_{leaf} variability and then evaluate the relationship between rainfall and Δ_{leaf} in *M. quinquenervia* by determing Δ_{leaf} from an 11-year long monthly collection of leaves. Finally, we identify factors that lead to leaf preservation and map wetlands that have the potential to preserve sub-fossil leaves of *M. quinquenervia*.

Carbon isotope discrimination in leaves

Δ_{leaf} reflects the net photosynthetic carbon isotope fractionation relative to the $\delta^{13}C$ of atmospheric CO_2. Δ_{leaf} varies among plants with different photosynthetic pathways; i.e. C_3, C_4 and CAM. *M. quinquenervia* utilises the C_3 photosynthetic pathway (Kattge *et al.*, 2011). Within C_3 plants, environmental variables such as the availability of water, pCO_2 and irradiance can further modify Δ_{leaf} (Cernusak et al., 2013; Shubert and Jahren, 2012). The effects of irradiance are particularly evident in closed canopy environments (Graham *et al.*, 2014).

Relationship between Δ_{leaf} *and precipitation in* C_3 *plants*

Plants preferentially fix ^{12}C over ^{13}C during photosynthesis. This preference is expressed as carbon isotope discrimination and is defined as:

$$\Delta_{leaf} = \frac{(\delta_{atm} - \delta_{leaf})}{1 + (\delta_{leaf}/1000)} \qquad (1)$$

Where δ_{atm} and δ_{leaf} represent the $\delta^{13}C$ of atmospheric CO_2, and the leaf, respectively (Farquhar et al., 1982).

The most widely used model for carbon isotope discrimination during C_3 plant photosynthesis, developed in Farquhar *et al.* (1982), explains carbon isotope discrimination as the result of the isotopic effects of diffusion of CO_2 through stomata and carboxylation by RuBisCo during photosynthesis, such that:

$$\Delta_{leaf} = a + (b - a)(\frac{c_i}{c_a}) \tag{2}$$

where *a* and *b* are constants that represent fractionation due to diffusion (4.4‰) and carboxylation (27-29‰), respectively; c_i and c_a, represent the partial pressure of CO_2 (pCO_2) inside the leaf, and the ambient atmosphere, respectively (Cernusak *et al.*, 2013, Farquhar *et al.*, 1989). Although more complicated models exist, this model sufficiently describes photosynthetic carbon isotope discrimination in many different settings (Cernusak, *et al.* 2013). In this model carbon isotope discrimination is entirely a function of the c_i/c_a ratio.

Key factors determining c_i/c_a are the supply of CO_2 to the leaf and the photosynthetic demand for CO_2. Supply of CO_2 is a function of stomatal conductance (i.e. the rate of passage of gases through the stomata). Stomatal conductance also controls the flux of water out of the leaf via transpiration, creating a trade-off between water loss and CO_2 uptake. As moisture availability increases, plants can afford to increase stomatal conductance, increasing c_i/c_a and increasing carbon isotope discrimination (Cernusak *et al.*, 2013; Diendendorf *et al.*, 2010). On the demand side of the equation, light limitation can lower the photosynthetic fixation of CO_2 leading to an increase in c_i, thereby increasing c_i/c_a and Δ_{leaf}. Our study species, *M.*

quinquenervia, lives in relatively open wetland environments and forms the uppermost canopy layer. As a result, canopy shading is unlikely to have a large influence on Δ_{leaf}, but self-shading could have an effect and is considered in this study.

Furthermore, Δ_{leaf} can be altered by the concentration of carbon dioxide in the atmosphere ($p\text{CO}_2$) (Schubert & Jahren, 2012). This relationship is described by the hyperbolic equation (Schubert and Jahren, 2012):

$$\Delta_{leaf} = \frac{[(28.26)(0.21)(pCO_2+25)]}{[28.26 + (0.21)\times(pCO_2+25)]} \ . \qquad (3)$$

In this equation, the value of 28.26 represents the asymptote or maximum carbon isotope discrimination as determined empirically through experiments on a number of plants grown under a wide range of $p\text{CO}_2$ and high nutrient and water availability (Schubert and Jahren, 2012).

The relationship between $p\text{CO}_2$ and Δ_{leaf} is steepest at low $p\text{CO}_2$ concentrations. Hence, the $p\text{CO}_2$-induced change in Δ_{leaf} through the last glacial-interglacial cycle is argued to exceed 2‰ (Schubert and Jahren, 2012). In the Holocene, the change in Δ_{leaf} resulting from the shift in $p\text{CO}_2$ from 251 ppm at the start of the Holocene to 281 ppm at 600 year BP (Monnin *et al.*, 2004) would result in a shift of approximately 0.6‰ in Δ_{leaf}. This change is noteworthy compared to those resulting from moisture stress, with a 0.6‰ shift in Δ_{leaf} equivalent to that induced by an increase in mean annual rainfall from 1000 to 1285 mm based on the relationship observed for evergreen angiosperms in Diefendorf *et al.* (2010).

Global rainfall-carbon isotope relationships

Global syntheses of rainfall-Δ_{leaf} relationships demonstrate the potential to quantitatively infer past rainfall from (sub)fossil leaves (Diefendorf *et al.*, 2010, Kohn, 2010). Diefendorf *et al.* (2010) found a statistically significant positive relationhip between mean annual rainfall ($\log_{10}$ transformed) and Δ_{leaf} (r^2=0.55, p<0.0001). Notably, this global relationship masks variability at the continental scale. For example, there is no statistically significant relationship exhibited in Europe (r^2=0.025, p=0.125). Kohn (2010) developed a more complex model that includes the influence of altitude and latitude. It showed similarly strong relationships between rainfall and Δ_{leaf}, particularly below ~1700 mm a^{-1}, with a largely constant isotopic discrimination at higher rainfall amounts.

While these meta-analyses point to the potential to reconstruct rainfall from Δ_{leaf}, only ~50% of the variance (on log scales) in isotope discrimination is explained by mean annual precipitation (Diefendorf *et al.*, 2010, Kohn, 2010). Substantial Δ_{leaf} variation between different species has been observed under the same water availability regime in natural (Cernusak *et al.*, 2013) and experimental (Cernusak *et al.*, 2009) settings. The between-species variability in rainfall-Δ_{leaf} relationships is so large that it has been argued Δ_{leaf} cannot be used to infer past precipitation from a mixed leaf fossil assemblage (Freeman *et al.*, 2011).

Rainfall-carbon isotope relationships in Australia

Statistically significant relationships between rainfall and $\delta^{13}C_{leaf}$ of plant communities (Stewart *et al.*, 1995), $\delta^{13}C_{leaf}$ of C_3 grasses (Murphy & Bowman, 2009) and $\delta^{13}C$ in charcoal (Turney, 2012) have been demonstrated in Australia across broad spatial gradients. By contrast, Schulze *et al.* (1998) noted that Δ_{leaf} did not increase above 475 mm in mixed species samples from Northern Australia, and only seven out of thirteen *Eucalyptus* species from the same study region showed significant (positive) relationships between Δ_{leaf} and rainfall (Miller *et al.*, 2001). These studies were undertaken across broad spatial gradients, mirroring research in other locations (e.g. Ehleringer, 1993, Weiguo *et al.*, 2005). Spatial sampling regimes have the potential to overestimate the explanatory power of rainfall due to co-variation with confounding factors (e.g. changes in soil type and moisture, elevation and aspect) and spatial autocorrelation (see discussion in Telford & Birks, 2009). In contrast to previous mixed species and spatial study designs, this study circumvents these uncertainties by sampling leaves of *M. quinquenervia* over a period of 11 years at a single site.

Spatial distribution of Melaleuca quinquenervia

M. quinquenervia is native to the eastern coastal margin of Australia (between 8–34°S) and to Papua New Guinea (PNG) and New Caledonia (Fig. 1a). It is generally found proximal to wetlands as its seeds have an obligate need for moist substrates for propagation, although mature trees will survive in locations with low soil moisture (Doran & Turnbull, 1997). *M. quinquenervia* has a shallow root system, with over 80% of the root biomass occurring in the top 15 cm of the soil in our Carbrook Wetlands study site (Bradley, 1996). In Australia and PNG, *M. quinquenervia* mostly grows at altitudes of less than 100 m above sea level, while in New Caledonia, where *M. quinquenervia* is the dominant savannah tree (Ibanez *et al.*, 2013), it grows at altitudes up to 1000 m. The mean maximum temperature of the hottest month over *M. quinquenervia*’s range in Australia is 26–34°C (Doran & Turnbull, 1997), while the mean minimum temperature of the coolest month is between 4 and 20°C (Doran & Turnbull, 1997). The species occurs across a wide rainfall gradient, ranging from 400 to 4000 mm per annum (Fig. 1b). *M. quinquenervia* is an invasive species in the Florida Everglades, USA and a substantial literature discusses the reasons for, and possible solutions to, this problem (Dray Jr *et al.*, 2006).

Regional Setting

Our study analyses *M. quinquenervia* from two locations in south-east Queensland, Australia: Carbrook Wetlands (Fig. 1c), where a long-term leaf collection was undertaken, and Swallow Lagoon, North Stradbroke Island, where we analysed leaf and stand-scale variation in Δ_{leaf}. Carbrook Wetlands are located in the catchment of Native Dog Creek and the Logan River Floodplain (27.690 S, 153.276 E) (Fig. 1c). *M. quinquenervia* forest occupies an area of 164 ha in the wetland, which is described by Greenway (1994). Mean annual rainfall at nearby Mount Cotton Farm (Australian Bureau of Meteorology station number 040460), 7.2 km north of Carbrook Wetlands, (Fig. 1d) is 1289 mm (annual rainfall range: 634–2499 mm, n=34). Mean annual maximum temperature at the Logan City Water Treatment Plant, approximately 5 km west of Carbook Wetland (Bureau of Meteorology station number 40854) is 26°C, with the highest mean monthly maximum temperature in January of 29°C and the lowest mean monthly maximum of 21.4°C in July.

Swallow Lagoon (27.499 S, 153.455 E) is a small (<1.5 ha) relatively deep (5.8 m maximum depth) perched freshwater lake (Marshall & McGregor, 2011) (maximum recorded elevation of the lake surface: 154 m AHD, April 2011) on North Stradbroke Island, a large (285 km^2) sand island in Moreton Bay, Queensland (Barr *et al.*, 2013). Swallow Lagoon is fringed by *M. quinquenervia*, which forms a narrow ring around the lake at a distance of less than 10 m from the water's edge and in water up to 0.5 m deep (in March 2014).

Material and Methods

The weight of *M. quinquenervia* litterfall and its carbon isotope discrimination was analysed from a leaf litter tray on the Carbrook Wetlands floodplain, 100 m from Native Dog Creek. The litter tray was located in a position which had 2175 trees ha^{-1}, with a mean height of 18.6 m (±4 m, 1 s.d.) and a mean diameter at breast height of 17.8 cm (±9 cm 1 s.d.) (Greenway, 1994). The density of the trees, and collection of windblown leaves of species which did not overhang the tray, indicates the tray integrates leaf fall from a number of canopy trees, rather than a single individual (Greenway, 1994). Litterfall was collected from a raised 0.25 m^2 (0.5 m × 0.5 m) leaf litter tray at *ca.* four-weekly intervals between April 1992 and July 2003. We thereby extend the record of Greenway (1994) through provision of more recent data (April 1994–July 2003) and additional analyses. Hereafter, this data set is referred to as the "temporal" data set, with the sampling interval described herein as "monthly". Litterfall was sorted into a number of different components (including leaves divided into "young" – identified by their covering of silky white hairs which disappear as the leaf matures – and "total" categories), oven dried at 40°C and weighed. These data provide an understanding of the phenology of *M. quinquenervia* and, in particular, identify the period of new leaf formation and highest leaf production. Leaves were stored in paper bags until 2011. From each monthly collection, a 1 cm slice from the leaf tip was sampled from a mean of nine leaves for δ^{13}C analysis.

We examined variation in *M. quinquenervia* Δ_{leaf} at the leaf, tree and stand scale from trees fringing Swallow Lagoon. Discrimination within a leaf may vary with distance from the vein due to increased diffusive pathway length (Seibt *et al.*, 2008). The leaves of *M. quinquenervia* have five longitudinal veins (as indicated by the species epithet), which may reduce the effects of diffusive length. We nevertheless undertook experiments to assess within leaf variation in Δ_{leaf}. In these experiments, leaves were cut into 12 sections (Fig. 2) and Δ_{leaf} determined on each subsample. At the stand scale, Δ_{leaf} may be influenced by factors including the leaf's position in the canopy (Marshall *et al.*, 2007), orientation on the tree (as has been observed by Lockheart *et al.*, 1998) and edaphic factors such as temperature and water availability (Diefendorf *et al.*, 2010, Kaplan *et al.*, 2002). Hence, we examined the difference between north facing (more sun exposed) and south facing (more shaded) leaves on four trees and the variation between these trees growing at positions 1 m outside the lake, at the lake edge, and at 3 m and 6 m inside the lake.

All leaf samples were washed in deionised water, freeze dried and milled to a fine powder prior to $\delta^{13}C$ analysis. $\delta^{13}C$ analyses were performed on a Costech Elemental Analyser coupled to a VG TripleTrap and Optima dual-inlet mass spectrometer, with $\delta^{13}C$ values reported on the VPDB scale using a within-run laboratory standard calibrated against NBS-18, NBS-19 and NBS-22. Replicate analysis of well-mixed samples indicated a precision of $\pm <0.1‰$ VPDB (1 s.d.).

Δ_{leaf} was calculated with equation 1 using $\delta^{13}C_{atm}$ derived from an average of monthly values for the two years prior to leaf sample collection as measured at the Mauna Loa Observatory, Hawaii, by the United States of America's NOAA Earth System Research Laboratory, Global Monitoring Division (www.esrl.noaa.gov/gmd). We also derived a relationship that removes the effect of pCO$_2$ on Δ_{leaf} by calculating the difference between Δ_{leaf} predicted from pCO$_2$ (equation 3) and the Δ_{leaf} from the carbon isotope values measured in our leaves (equation 1). This calculation used

$p\mathrm{CO}_2$ data from Mauna Loa Observatory for 2 years prior to dates of leaf collection. This difference between $p\mathrm{CO}_2$-predicted and measurement-based discrimination is referred to as the discrimination anomaly.

While the primary aim of our study is to investigate the relationship between *M. quinquenervia* Δ_{leaf} values and rainfall, some studies (e.g. Diefendorf *et al.*, 2010) have shown that other climate variables (e.g. temperature) may be of secondary importance. Hence, we investigated the degree to which variability in *M. quinquenervia* Δ_{leaf} was explained by a range of potential environmental variables, namely: rainfall, minimum and maximum daily temperature, pan evaporation, precipitation divided by evaporation (hereafter P/E), solar radiation - hereafter termed “radiation”, vapour pressure and relative humidity at both maximum and minimum temperature. Precipitation data were taken from the Mount Cotton Farm rainfall station (Fig. 1). Where no rainfall data were available for a particular month (*n*=4), we estimated rainfall amount from a linear relationship, developed from the remaining study period, between that month’s rainfall and that of the adjacent month most correlated to the month with a single missing value. Other climate data were derived from the Queensland Department of Science, Information Technology and Innovation’s Scientific Information for Land Owners dataset which derives synthetic climate data interpolated across space from variables measured at climate stations (Jeffrey *et al.*, 2001).

Our analyses of the relationships between climate and Δ_{leaf} were undertaken using two temporal data sets. The first determines the relationship between the individual Δ_{leaf} values collected at monthly intervals and climate data for which composite means were calculated for a range of time windows prior to the month of leaf collection. Climate data in the year immediately prior to leaf fall was excluded because very few young (<1 year old) leaves were collected in the tray (Fig. 3). Thus, the time composites encompassed periods of 1 to 2 years, 1 to 3 and 1 to 4 years prior to leaf fall. For the second set of analyses, annual mean Δ_{leaf} was calculated for each hydrological year (November 1st to October 31st). Annual mean values were compared to both mean rainfall for the hydrological year, and individual month's rainfall, again composited for periods of between 1-2 years and 1-4 years before leaf collection.

In order to identify sites where *M. quinquenervia* leaves may be preserved we identified where this species grows close to wetlands that may favour preservation. Vegetation associations (known as regional ecosystems; Neldner *et al.*, 2012) with *M. quinquenervia* as the dominant or sub-dominant canopy tree were mapped where they fell within 30 m of lacustrine or permanent palustrine wetlands using the Queensland Government's Regional Ecosystem Description Database (www.qld.gov.au/environment/plants-animals/plants/ecosystems/about/#redd) vegetation associations. The location of the wetlands was identified from satellite imagery, with all wetlands < 8 ha classified as palustrine and the distinction between lacustrine or palustrine for wetlands > 8 ha based on whether open water (lacustrine) or vegetation (palustrine) dominated the site (Neldner *et al.*, 2012). To further identify sites of, and conditions favouring preservation, we collected sediment cores from 10 lakes and swamps on North Stradbroke and Fraser Islands using piston (Livingstone, 1955) or side sampling (Jowsey, 1966) corers. Continuous sediment sections (between 1 and 5 cm in width) from these cores were sieved with a 250 μm sieve, with the retained material inspected for *M. quinquenervia* leaves. Lastly, at Swallow Lagoon we used a YSI 6920 multi-parameter sonde to determine the lake water dissolved oxygen profile approximately monthly between June 2011 and May 2012, while historical dissolved oxygen data from Brown Lake, North Stradbroke Island (see supporting information S3) are also discussed.

Results

Phenology of Melaleuca quinquenervia

M. quinquenervia produces new leaves throughout the year but with marked seasonality in production. Peak leaf production (young leaf fall) commences in June and is greatest in August–November (Fig. 3). It is unlikely that young leaf fall is related to wind stress since the period of maximum abscission of older leaves frequently occurs after the abscission of young leaves in the seasonal cycle (Fig. 3). The small number of young leaves collected (<1% of leaf weight over the sampling period) indicates that most leaves remain on the tree for over one year.

Intra-leaf Δ_{leaf} variation and variation within a stand

In the three leaves analysed, the within-leaf variability of Δ_{leaf} was less than 1‰. The mean Δ_{leaf} for the 12 sub-samples from the three leaves was 23.82‰ ± 0.29, 24.48‰ ± 0.18 and 23.85‰ ± 0.22 (± 1 standard deviation [s.d], Fig. 4). Moreover, the leaf tips – equivalent to the leaf fraction sampled in our temporal sampling – have Δ_{leaf} values within the range measured for the other parts of the leaf (Fig. 4a). We also found no systematic differences between central and marginal leaf Δ_{leaf} for individual leaves (see Fig. 4b). The Δ_{leaf} of north (mean=24.40‰, s.d.=1.16‰, n=4) and south (mean=24.44‰, s.d.=0.83‰, n=4) facing samples of four trees at Swallow Lagoon exhibited no systematic difference (Fig. 5).

Temporal relationship between rainfall and Δ_{leaf} of M. quinquenervia

The Δ_{leaf} of *M. quinquenervia* collected from April 1992 to July 2003 in Carbrook Wetlands ranged from 20.46‰ to 24.90‰, (mean: 22.63‰, s.d.: 0.9‰, *n*=137) (supporting information S1). Carbon isotope discrimination systematically increased in 1996 (Figure 6), with values before August 1996 rarely exceeding 22.50‰ (mean: 21.89‰) and after this time mostly exceeding 22.50‰ (mean: 23.09‰). Importantly, the shift in Δ_{leaf}, corresponds to an increase in rainfall at nearby Mount Cotton Farm in the 24 months prior to leaf fall (Fig. 6).

The relationship between *M. quinquenervia* Δ_{leaf} and climate was first examined using the monthly Δ_{leaf} data. All the climate variables analysed, with the exception of maximum temperature, were significantly, but weakly, correlated with monthly Δ_{leaf} data for at least one of the time periods considered (1-2, 1-3 and 1-4 years before leaf fall) (Table 1). However, spurious correlations can emerge from correlation of biological data with multiple climate variables over multiple timescales. This is particularly problematic in this study as *M. quinquenervia* leaves remain on the tree for a variable period of time (between 1-4 years). Therefore, each monthly collection provides a time-averaged sample spanning up to four years prior to leaf collection, resulting in a need to examine relationships to climate over varied time intervals. In addition, unlike the litter collection, natural accumulations of leaves in sediments are mixed over multiple years, calling for a calibration that examines climate relationships relevant to that coarser sampling resolution. As a result of these considerations, we focus the remaining analysis on the relationship between climate and Δ_{leaf} pooled into yearly means.

When the relationship between climate and annual mean Δ_{leaf} is derived, only two climate variables exhibited significant relationships to Δ_{leaf}: rainfall 1-4 years before leaf collection and radiation 1-4 years before leaf collection (Table 2). Rainfall and radiation are significantly correlated (r^2=0.54, p=0.011). Based on this outcome, we developed rainfall calibrations that can be applied to sub-fossil records (Table 3). One relationship is based directly on Δ_{leaf} and one takes into account the effect of pCO$_2$ on Δ_{leaf} (Schubert & Jahren, 2012) (see methods). There was little difference in the strength of the isotope-rainfall relationship based on Δ_{leaf} and discrimination anomaly (Fig. 7), due to the linear increase in atmospheric pCO$_2$ through the study period (www.esrl.noaa.gov/gmd). However, given the influence of pCO$_2$ variation on Δ_{leaf} through time, the application of the model that accounts for this effect is preferable. The relationship between rainfall 1–4 years before leaf collection and the anomaly (which accounts for the influence pCO$_2$ on Δ_{leaf}) is slightly stronger (r^2=0.67 p=0.002, n=11) (Fig. 7) than that between Δ_{leaf} and rainfall over the same period (r^2=0.64, p=0.003, n=11).

Discussion

Isotope systematics of leaves

Analysis of intra-leaf variability shows no discernible difference in Δ_{leaf} values between the leaf tip and other parts of the leaf (Fig. 4). This suggests the leaf tip Δ_{leaf} used in our temporal study represents the whole leaf Δ_{leaf}. Additionally, it appears any given leaf fragment (e.g. those occurring in sub-fossil deposits) can provide a representative whole leaf Δ_{leaf} value for *M. quinquenervia*. Furthermore, our analysis shows leaf position on individual trees (north vs south facing), and the position of the trees relative to the water's edge do not bias the isotopic analysis as there are no systematic differences between leaves or trees in different positions. Therefore, leaf position on the tree, and the tree's location relative to the water's edge, should have a minimal impact on Δ_{leaf}.

Relationship between M. quinquenervia *Δ_{leaf} and climate*

Our analysis of monthly Δ_{leaf} for *M. quinquenervia* for an 11 year period reveals significant but weak correlations with all of the climate variables examined on at least some of the time intervals prior to leaf collection. The numerous weak correlations reflect the complexity of the relationship between climate and Δ_{leaf} on short timescales. However, when the climate and leaf data are pooled into yearly means, a process akin to filtering the climate data, then only rainfall and radiation (in the 1-4 years preceding leaf fall) are significant. The significant relationship (r^2=0.64, p<0.003, n=11) between Δ_{leaf} and rainfall (Table 2; Fig. 7) mirrors observations made at the scale of individual plant species (Van de Water *et al.*, 2002), communities (Prentice *et al.*, 2011, Stewart *et al.*, 1995) and the globe (Diefendorf *et al.*, 2010, Kohn, 2010). This study, however, assesses the relationship between Δ_{leaf} and rainfall at a single location, rather than across a mean annual precipitation gradient as used in most previous studies. Hence, our analysis represents a robust assessment of rainfall-isotope relationships as it negates effects associated with (spatial) autocorrelation frequently encountered in calibration data sets (Telford & Birks, 2009). The strong relationship between this species and rainfall is in contrast to that observed in other studies (Miller *et al.*, 2001, Schulze *et al.*, 1998, Van de Water *et al.*, 2002). *M. quinquenervia*'s adaptation to a wide range of environments with varying moisture availability (see Fig. 1b) may contribute to the strong relationship observed.

The potential to quantify past tropical and subtropical rainfall

A review of the locations in eastern Australia where *M. quinquenervia* leaves may preserve highlights enormous potential to establish quantitative rainfall reconstructions from the Δ_{leaf}-rainfall relationships described herein. Our analysis shows there are over 200 permanent wetlands in the Australian state of Queensland where *M. quinquenervia* grows within 30 m of the wetland (Fig. 8).

To investigate whether *M. quinquenervia* leaves are preserved in some of these sites, we recovered sediments from ten lakes and swamps on the sand islands North Stradbroke Island and Fraser Island (Table 3). On these islands, three out of the ten wetlands surveyed preserved leaves of *M. quinquenervia* and two also preserved leaves of *Eucalyptus* (Table 3). *M. quinquenervia* has highly resistant sclerophyllous leaves (Li *et al.*, 2009), which favours their preservation, however sclerophylly does not ensure preservation since there was no preserved material found at a number of other sites we sampled (Table 3). In addition, no leaves were preserved in sediments of Lachlan Nature Reserve swamp, Sydney, Australia (R. Hamilton pers. comm.), a wetland which has no permanent standing water but has *M. quinquenervia* as the current canopy dominant (Hamilton & Penny, 2015). As a result, we hypothesise the preservation of leaves is dependent on anoxia, which reduces chemical and biological breakdown (e.g. by macroinvertebrate "shredders" Li *et al.*, 2009). Anoxia in these low nutrient lake systems is likely to result from their physical characteristics, in particular the ratio of lake surface area to both depth and water volume, and the degree of protection afforded from wind driven overturning by the surrounding terrain (including vegetation) (Hondzo & Stefan, 1996). As an example, monitoring the deepest area of Swallow Lagoon at approximately monthly intervals between June 2011 and May 2012 found that it experiences prolonged anoxia (near bed dissolved oxygen typically < 1 ppm; supporting information S3) for most of the year due to its sheltered position and small surface area (< 1.5 ha). By contrast, larger more exposed lakes on North Stradbroke Island typically have higher oxygen concentrations at depth. Blue Lake never experienced anoxia for the period 1996–2002 (Barr et al. 2013) and lakebed anoxia was only recorded once in Brown Lake for the period 1992-2001 (*n*=47; supporting information S3). Neither of these sites preserved leaves (Table 3). Beyond our investigations, there are large numbers of potential sites for preservation of *M. quinquenervia* in Queensland, Australia (see Fig. 8). Outside Australia *M. quinquenervia* has been observed on the margins of palaeoecological sites in New Caledonia (Stevenson *et al.*, 2001).

Conclusions

The annual mean carbon isotope discrimination of *M. quinquenervia* leaves collected over an 11-year period exhibits a statistically significant relationship to rainfall. In the modern day, the effect of $p\mathrm{CO}_2$ is largely negligible for our samples; however, for application to the fossil record, changes in $p\mathrm{CO}_2$ even during the Holocene has an important effect (Schubert & Jahren, 2015). Variation within leaves and within the canopy is not systematically offset and is small relative to the overall rainfall-isotope relationship. Therefore the utility of Δ_{leaf} in *M. quinquenervia* as a proxy for past rainfall variability has great potential in locations where leaves are preserved.

An analysis of the distribution of *M. quinquenervia* reveals a large number of wetlands across the Australian eastern seaboard that may contain sub-fossil leaves within their sediments. A survey of ten lakes on North Stradbroke Island and Fraser Island found three lakes with abundant preservation within the sediments. The rainfall-Δ_{leaf} relationship, widespread distribution and demonstrated preservation of *M. quinquenervia* leaves, indicate substantial potential for reconstructing past variability in tropical and subtropical precipitation.

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Supporting information captions

S1: *Melaleuca quinquenervia* Δ_{leaf} from Carbook Wetland April 1992-July 2003.

S2: Monthly and annual rainfall from Mt Cotton Farm

S3: Swallow Lagoon and Brown Lake, North Stradbroke Island dissolved oxygen concentrations

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Table 1. Coefficient of determination for significant (*p*<0.01) relationships between monthly Δ_{leaf} and climate variables over different time intervals before leaf collection (blc). RHmaxT and RHminT=relative humidity at maximum and minimum temperature, respectively. n/s=not significant.

Climate variables	Coefficient of determination of relationship with monthly Δ_{leaf}		
	1 - 2 years blc	1 - 3 years blc	1 - 4 years blc
Evaporation	0.13	0.10	n/s
Minimum temperature	0.30	0.28	0.15
P/E	0.19	0.28	0.18
Radiation	0.18	0.23	0.26
Rainfall	0.17	0.28	0.19
RHmaxT	0.15	0.16	0.16
RHminT	n/s	n/s	0.06
Vapour Pressure	0.19	0.18	0.14

Table 2. Relationship between Δ_{leaf} and climate variables for 1–2, 1–3 and 1–4 hydrologic (Nov-Oct) years before leaf collection (blc). Only significant (*p*<0.01)

variables are displayed. Rainfall data are expressed as yearly mean (mm) while radiation data are daily mean (MJ/m^2).

Variable	**Relationship**	**Linear regression**
Annual average rainfall 1–4 years (365–1460 days) blc	r^2=0.64, p=0.003	y = 0.004x + 17.936
Radiation 1–4 years (365–1460 days) blc	r^2=0.57, p=0.008	y = -2.1557x + 62.815

Table 3. Wetlands with *M. quinquenervia* growing on the wetland surface or around the margin that we investigated for leaf preservation. For additional background information about North Stradbroke Island (NSI) wetlands see Marshall *et al.* (2011).

Wetland	**Site type**	**Location (and island)**	**Reference (for lake characteristics)**	**Water depth at time of sampling**	**Wetland area**	**Leaves recovered?**
18 Mile Swamp (pools)	Freshwater coastal swamp	27.526444 S 153.496778 E (NSI)	Mettam *et al.* (2011)	2.3 m	0.1 ha	No
Brown Lake	Perched lake	27.488291 S 153.434740 E (NSI)	Mosisch and Arthington (2001); Marshall *et al.* (2011)	6.1 m	46 ha	No
Blue Lake	Groundwater window lake	27.518056 S 153.477417 E (NSI)	Barr *et al.* (2013); Marshall and McGregor (2011); Marshall *et al.* (2011)	7.2 m	10.3 ha	No
Fern Gully Lagoon	Perched palustrine wetland	27.25053 S 153.27663 E (NSI)	Marshall *et al.* (2011)	1 m	7.3 ha	No
Swallow Lagoon	Perched lake	27°.41772 S 153°.46118 E (NSI)	Mosisch and Arthington (2001); Marshall and McGregor (2011); Marshall *et al.* (2011)	5.7 m	<1.5 ha	Yes. *Eucalyptus* leaves also preserved
Welsby Lagoon	Perched palustrine wetland	27.43717 S 153.45010 E (NSI)	Moss *et al.* (2013); Marshall *et al.* (2011)	1.2 m	19.25 ha	No
Barga Lake	Perched lake	25.5 S 153.05 E (Fraser Island)	Bayly *et al.* (1975)	0.8 m*	7.0 ha	No
Basin Lake	Perched lake	25.466667 S 153.05 E	Bayly *et al.* (1975)	7.5 m	3.8 ha	Yes

		(Fraser Island)				
Benaroon Lake	Perched lake	25.516667 S 153.05 E (Fraser Island)	Bayly *et al.* (1975)	5.7 m	83 ha	No
Jennings Lake	Perched lake	25.494 S 153.055 E (Fraser Island)	Bayly *et al.* (1975)	4.1 m	12.2 ha (open water area)	Yes. *Eucalyptus* leaves also preserved

*deepest part of the lake not sampled.