Canopy change through the Cenozoic in South Eastern Australia

Thesis submitted in accordance with the requirements of the University of Adelaide for an Honours Degree in Geology

Benjamin James Birch

November 2015

CANOPY CHANGE THOUGH THE CENOZOIC IN SOUTH EASTERN AUSTRALIA

CENOZOIC CANOPY CHANGE

ABSTRACT

Reconstructing canopy closure is difficult, and has up until recently only been done through interpretation of cuticular morphology. However, along with the morphology, isotope characteristics preserved in the leaves have enabled the deduction of the "canopy effect", and thus the able to reconstruct the closure of ancient forests. Australia has had rich and unique development since its separation from Antarctica and its flora has developed from closed canopy tropical rainforests, of the Eocene, to its open arid hummock grasslands of the present day. To assess the canopy change from the Eocene through to the Miocene, we employed the carbon isotopic data from leaf fragments from two sites, Anglesea (Victoria) from the Eocene, and Kiandra (New South Wales) from the Miocene, and compared them to present day carbon isotopic data of open and closed canopy forests. There were two assessments conducted on the sites, and individual leaf and a dispersed cuticle, to validate whether dispersed cuticle is reflective of single leaf specimens for the deposit. The mean individual leaf values of the fossil sites show that they are significantly more depleted in ^{13}C, resulting in the larger Δ_{leaf} values. Anglesea has a larger mean value at 28.01 ± 0.52‰, with Kiandra having a lower value of 27.45 ± 0.51‰. The range of isotopic values, for the two sites are 6.10‰ and 6.2%. An additional test in Anglesea showed that there is a large influence in the dispersed cuticle from gymnosperms. In Modern closed canopy, tropical rainforests have mean isotopic ranges of approximately 5.8‰, with mean values of 22.59‰. Whereas modern open canopy temperate forests have isotopic ranges of approximately 4.8‰, with mean values of 21.08‰. So, upon inspection the Anglesea and Kiandra sites are both similar to tropical rainforests. An additional study was conducted of dispersed cuticle, across the Eocene-Miocene interval, for 13 sites. The results varied, showing a decrease in canopy structure during the Oligocene from the Eocene, followed by the closed canopy Miocene.

KEYWORDS

Canopy closure, Australia, Cenozoic, Anglesea, Kiandra, Stable isotope geochemistry

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

INTRODUCTION

Forests are the dominant terrestrial ecosystem of Earth, and are found at most latitudes, from the boreal forests near the poles (as far as 70°, north of the equator) to the rainforests near the equator. Forests often have several distinct vertical layers, from the floor to the upper most layer called the canopy. Closed forest canopies, such as those found in tropical rainforests, are thought to have originated with the rise of angiosperms (flowering plants) (van der Merwe and Medina, 1991). It is difficult to determine the structural characteristics of forest canopies in the fossil record using standard palaeobotanical techniques (Boyce and Lee, 2010)**,** and so the structural changes in forest canopies in response to paleoclimate changes remains understudied.

However, Graham et al (2014) have pioneered a new geochemical method of accurately profiling the canopy structure of modern forests using the carbon isotope signatures of forest leaf litter. Applying this technique to the fossil record may have important implications for understanding paleoclimate, as well as how global forest systems may respond to future climate changes. A proxy is used to indirectly measure the character of the canopies structure, with stable isotope ratios contained in fossil leaves. The leaves have preserved carbon isotopic signatures that indicate the environment that they grew in. Carbon isotopic gradients allow characteristics, such as closure of canopies to be distinguished (Graham et al., 2014; van der Merwe and Medina, 1991).

Analysis of isotope fractionation in plant tissue is one of the key techniques available in the reconstruction of palaeoenvironments (Barbour, 2007). Carbon isotope discrimination (Δ) is defined as the depletion of ^{13}C during any process preferring the lighter isotopologue, following the equation:

$$\Delta = (\delta C^{13}{}_{CO_2} - \delta C^{13}{}_{leaf})/(1 - \delta C^{13}{}_{leaf}/1000) \quad (1)$$

Where $\delta^{13}C$ is the ratio of $^{13}C/^{12}C$ in the sample, compared to the ratio in the international standard, a Cretaceous belemnite formation at Peedee in South Carolina, USA (Cernusak et al., 2008). The $\delta^{13}C_{CO2}$ is the carbon isotope composition of the atmosphere, and $\delta^{13}C_{leaf}$ is the bulk carbon isotopic composition measured in plant tissue. As shown by the equation, as the $\delta^{13}C$ values more negative the Δ will increase.

The carbon isotope discrimination of plant tissues are influenced by many biochemical and biophysical properties, some of the key effects include: photosynthetic pathways, such as C_3, C_4 and CAM; and whether they are an angiosperms or gymnosperms. Furthermore, different tissue types, such as lignin and polysaccharides, have subtle differences in their isotopic signatures (Arens et al., 2000; Benner et al., 1987; Cerling et al., 1997; Condon et al., 1990; Diefendorf et al., 2010; Graham et al., 2014).

Within C_3 plants carbon isotope discrimination of plant tissues are influenced by many geographic and environmental parameters. Altitude effects $\delta^{13}C$, shown by a decrease of Δ between 0.8 and 2.7‰ per km, and the higher the latitude the smaller the Δ. Tropical plants (low latitude) show average values of Δ around 21.8‰ and plants from subarctic and arctic lowland sites (high latitude) show values around 19.1‰ (Körner et al., 1991). Different light and temperature regimes affect the leaf thickness and thus affect the Δ values, i.e high temperature can lower the Δ value, similar to that of lower light attenuation (Cerling et al., 1997; Condon et al., 1990; Körner et al., 1991).

The carbon isotopic signatures of atmospheric CO_2, under closed canopies, become more negative, due to an increase of respiration from plants and the pCO_2 effect on Δ (Beerling et al., 1995; Schubert and Jahren, 2012), of $\delta^{13}C$ of atmospheric CO_2. The opening of stomates, in water excess environments, results in an increase of Δ within the plant tissue (Warren et al., 2001). High humidity, light stress, and atmospheric recycling of CO_2 play a significant part in closed canopy regimes, and results in what is known as the 'canopy effect'.

In forests, the 'canopy effect' is the gradient of $\delta^{13}C$ values in leaves of plants found at different layers of forests, where there is an increase in $\delta^{13}C$, and a decrease of Δ, from ground to canopy (van der Merwe and Medina, 1991). Explanations for this phenomenon includes recycling of ^{13}C-depleted CO_2 in the forest, fractionation during photosynthesis, such as low light levels, high humidity or high temperature environments (Graham et al., 2014; Jackson et al., 2012; Tieszen and Boutton, 1989; van der Merwe and Medina, 1991). A study conducted by Cerling et al (2004) in the dense tropical Ituri Forest, in the Democratic Republic of Congo, showed extreme depletion in $\delta^{13}C$ in leaves found in the understory. These values for plants from the sub-canopy to the gaps in the canopy, and the canopy top average −34.0‰, −30.4‰; and −29.0‰ respectively (Cerling et al., 2004).

Graham et al (2014) conducted a study in two modern forest types, a seasonal deciduous temperate forest, located in the Smithsonian Environmental Research Center (SERC) forest Maryland, USA; and a wet tropical forest, Bosque Protector San Lorenzo, Panamá, Central America. The Maryland site is a seasonal forest with its foliage being produced during the spring leaf flush were the canopy is open, and a and light attenuation of >99%, i.e. a light gradient with

less than 1% available light at the forest floor, is reached during the mid-summer growth period (Brown and Parker, 1994; Graham et al., 2014). The site in Panamá is an evergreen, moist tropical forest, with a persistent closed canopy, and light attenuation of >99%. The results for both the seasonal and tropical forests showed both an isotopic and a light attenuation gradient from the understory to the canopy.

The average $\delta^{13}C$ values show that there is an isotopic gradient from each of the forests. The average $\delta^{13}C$ values, from the seasonal forest, for the understory, the sub-canopy and canopy, are as follows: -30.5‰, -29.3‰, and -26.1‰, respectively (Graham et al., 2014). The tropical forest showed similar results for both the isotopic gradient, however they are more depleted in $\delta^{13}C$. The results, for the understory, to the sub-canopy to the canopy, are as follows: -32.8‰, -30.5‰, and -27.9‰, respectfully (Graham et al., 2014). Both sites show a consistent trend, with the most depleted $\delta^{13}C$ leaves located are in the understory, with the canopy leaves enriched in $\delta^{13}C$.

Both of the sites are distinguished by both their range of $\delta^{13}C$ values as well as their mean $\delta^{13}C$ values. The seasonal open forest has a mean and range value of -28.5‰ and 5.8‰, respectively, whereas, the Panamá forest shows more depleted and variable $\delta^{13}C$ values, with mean and range values of -30.2‰ and 9.6‰ respectively (Graham et al., 2014). Sample size is important when considering canopy structure, as the canopy effect is seen because of the range of isotopic values. If the sample size is not large enough the difference between an open canopy and a closed canopy is indistinguishable. To examine this (Graham et al., 2014) incorporated the $\delta^{13}C$ values from the two forests into a resampling model. All isotope data was then binned into vertical five-meter increments. The model then performed a bootstrap analysis of binned isotope data for the

forests. Each selected 'leaf' was assigned a random $\delta^{13}C$ value selected from the height bin. The model repeated sampling of discrete sizes, and then the process of assemblage collection repeated 2000 times resulting in 2000 simulated collection scenarios Graham et al (2014). These results predicted the isotopic range for the leaf assemblages from each forest, with as few as 50 randomly sampled leaves. Thus, by comparing the range of values, as well as the mean $\delta^{13}C$ values to equivalent modern open or closed canopy forests, it should be possible to reconstruct the canopy closure of an ancient forest using ~50 fossil leaves.

Fricke (2007) and Grein et al., (2010) showed that it is possible to reconstruct canopy structure in the fossil record. Fricke (2007), used the most common taxa along with paleosol samples in the Castle Rock region, Early Cenozoic (64.1Ma) in age, and located around Denver, CD, USA. This study showed that the carbon isotope ratios of the leaves sampled ranged from -28.8 to -24.8 ‰ (Beth, 2001; Fricke H., 2007). Grein et al., (2010), used fossil leaves belonging to three species: *Laurophyllum lanigeroides* (Lauraceae), *Daphnogene crebrigranosa* (Lauraceae) and *Rhodomyrtophyllum sinuatum* (Myrtaceae). The $\delta^{13}C$ of the fossil cuticles varies from –30 ‰ to –27 ‰ (Grein et al., 2010), which shows similar trend to Fricke (2007). The interpretation of these result were similar to Graham et al., (2014) study, that cause for these values as the result of the 'canopy effect'. Both studies were in regions during the Early Cenozoic in the northern hemisphere; Castle Rock in Denver, USA and Messel Pit, Germany, and did not consider the range of $\delta^{13}C$ values. During the Cenozoic, Australia changed dramatically from the early Eocene polar rainforests through to the present day arid outback. In saying this, canopy closure studies have never been applied to the southern hemisphere, let alone Australia.

The Indo-Australia plate is one of the fastest moving plates on the planet, 61 and 57 km/Ma during the Mid Oligocene and again in the Early Miocene, respectively, and as a result it has passed through a large number of latitudes (Cohen et al., 2013). At the start of the Cenozoic, Australia was connected to Antarctica and situated at the pole. After approximately 45 Ma Australia and Antarctica separated due to the opening of a mid ocean ridge (Feary et al., 1991; Quilty, 1994; Wei and Thierstein, 1991; Zachos et al., 2001), this and the opening of Drakes Passage caused the circum-polar current to form between 49-17Ma (Scher and Martin, 2006). Along with the Australia's change in position, its climate also changed dramatically.

During the Mid to Late Eocene the conditions were tropical (Quilty, 1994) and rainforests appeared across central Australia into the South Eastern part of the continent. The early Oligocene, marks the start of southern hemisphere icesheets, brought on by the circum-polar current, resulting in perihumid *(very wet, see table 1),* cool microthermal *(see table 1)* conditions (Martin, 2006; Quilty, 1994). Towards the late Oligocene the climate was considered to be more megathermal *(tropical, see table 1)* for the south eastern Australia (Quilty, 1994). Early Miocene is suggested to be similar to the late Oligocene, whereas the Mid Miocene was a very different interval. Mid Miocene showed evidence for large lakes and tropical rainforest lined rivers with increasing frequency of woodlands and grasslands (Martin, 2006; Quilty, 1994). During the Miocene, the climate was seasonal, with annual dry seasons (Macphail et al., 1994; Martin, 2006)

The vegetation of the epoch is suggestive of the climate changes during the Cenozoic. The Early and Middle Eocene was largely dominated by megathermal *(tropical, see table 1)* rainforests and

was considered warmer and wetter than the present day (Greenwood et al., 2003). Angiosperms dominated the rainforests with minor influences from gymnosperms, such as cycads, and conifers. The canopy structure is thought to be a closed canopy, which is similar to megathermal rainforests in the present day (Macphail et al., 1994; Martin, 2006; Martin, 1990). This is based upon the range of rainforest taxa as well as estimated rainfall. The Oligocene of Tasmania, the climate turned to microthermal *(cold winters, see table 1)*, with rainfall still high (Macphail et al., 1994; Martin, 2006; Martin, 1990). This cool wet change allowed the gymnosperms, specifically conifers, to dominate the forests during this epoch. During the Miocene the forests were a mosaic of megathermal and mesothermal *(temperate, see table 1)* plant taxa. Through the Miocene, angiosperms dominated the swamps, woodlands, and heath of south eastern Australia (Macphail et al., 1994; Martin, 2006; Martin, 1990). The rainforest taxa were isolated to small patches scattered around rivers and swamps; the gymnosperms also decreased in numbers but were still present in the woodlands (Martin, 2006)

Past studies of canopy structures, such as Fricke (2007) and Grein et al., (2010), did not consider the range of isotopic values in conjunction with modern day analogues. This study will address both the mean and range of isotopic values for fossil sites around south-eastern Australia. Using both individual leaf analysis as well as a possible new cheaper, less damaging method, using dispersed cuticle. Dispersed cuticle are structured fossilized phytoclasts, fossilized leaf fragments, and composed of resistant cuticle that have been separated from the leaves. During the Early Cenozoic, the Australian flora holds large diversities of both gymnosperms and angiosperms, with some fossil sites dominated by the gymnosperms. As such there will be gymnosperm cuticular fragments mixed with in the angiosperm fragments which may interfere

with dispersed cuticle measurements conducted in this study. This interference is also observed in modern day studies of angiosperms and gymnosperms show that they differ isotopically ($\delta^{13}C$) by up to 2%.

Here we seek to reconstruct canopy closure in south-eastern Australia during the Cenozoic, from approximately 50Ma (Middle Eocene) through to 15Ma (Middle Miocene). We employ two primary methods, the isotopic range and the isotopic mean of $\delta^{13}C$ values, to distinguish the degree of closure from a number of fossil sites. The range and mean of isotopic values were measured by sampling 50 individual angiosperm fossil leaf fragments, from two of the fossil sites, the first from Anglesea (New South Wales), aged at approximately 45-40Ma (Middle Eocene) the second from Kiandra (Victoria), aged at approximately 22-16Ma (Middle Miocene). Gymnosperms were also looked at in Anglesea, sampling about 20 in all, to test difference between angiosperms and gymnosperms found in the modern day. In addition the mean isotopic values were measured for an additional 13 fossil sites, by analysing the dispersed cuticle. The mean of the dispersed cuticle was tested by comparing the dispersed cuticle means with the mean generated by the individual leaf from the two sites. The dispersed cuticle sites are listed in tables 2 and 3 .We used the two sampling methods and isotopic methods to answer the following questions:

- Do stable isotope ratios reflect canopy closure from the Eocene to the Miocene using modern North and Central American analogues?
- Does $\delta^{13}C$ of dispersed cuticle reflect of the average from 50 angiosperm leaf specimens for a given deposit?

- If dispersed cuticle does not reflect the average from 50 angiosperm leaf specimens, what does it reflect? Why?

PREVIOUS GEOLOGICAL AND PALAEOBOTANICAL STUDIES

Table 1: Definitions for the different climate variable found in the text, adapted from Macphail (2007).

Temperature		
Category		**range**
Lower microtherm	Winters that are cold enough to ensure that snow will remain on the ground continuously	<10°C
Upper microthem		10-14°C
Lower mesotherm	Temperate – Sub Temperate: Coldest month that is colder than 18°C, but warmer than −3°C	14-18°C
Upper mesotherm		18-24°C
Megtherm	Equatorial – tropical, Every month has an average temperature of above 18°C	>24°C

Rainfall		
Category		**Range**
Wet-very wet	Very Wet - Wet	>1200 mm pa
±Wet	±Wet	900-1200 mm pa
±Dry	Sub-wet	600-900 mm pa
Very dry	Semiarid	300-600 mm pa
Arid	Arid	>300 mm pa

Symbol	Name	Place	Time Period	Location	Deposit Description	Depositional Environment	Elevation (m)	References
RP	Regatta Point (RP)	TAS	Early Eocene (52-51Ma*)	42" 10'S, 145" 20'E;	Early-Middle Pleistocene gravels with interbedded sandy silts, below are Pleistocene conglorates (within are RPA* and RPU** clasts) with early Eocene mudstones.	Widespread floodplains / lake (Lacustrine)	10	(Pole, 1992; Pole and Macphail, 1996; Wells and Hill, 1989)
NC	Nelly Creek (NC)	SA	Eocene (47-38Ma*)	29" 19'S, 137" 18'E; Southern shore of the Lake Eyre South	Unweathered carbonaceous laminated clay, silt and sand, may contain lenses of wood and leaf mats up to 50 cm thick	Meandering channel (Fluvial) to floodplain environment and the carbonaceous (leaf-bearing) clay in backwater swamps and shallow lakes on the floodplain	1100m	(Hill and Christophel, 2001)
A	Anglesea (A)	VIC	Eocene (45-40Ma*)	38" 25'S; 144"11'E; Alcoa open cut coal mine	Six laterally extensive lenses in mudstones and unconsolidated sands at 70cm below the ground surface	Meandering stream / oxbow pond (Fluvial) environment on a broad coastal plain that was adjacent to the Southern Ocean, given by the coal steams.	238	(Christophel et al., 1987; Macphail, 2007; Macphail et al., 1994)
GG	Golden Grove (GG)	SA	Middle Eocene (~45Ma*)	34" 47'S, 138" 43'E; Monier East Yatala Sand Pit	Fossiliferous clay lens	Possibly an oxbow lake (Lacustrine) surrounded by complex notophyll vine forest	120	(Hill, 1994; Macphail, 1994)
LA	Loch Aber (LB)	TAS	Mid Eocene (45-45*)	41" 02' S, 147" 58' E	Lens of laminated siltstone in gravel exposed in the Loch Aber tin mine in northeastern Tasmania	The siltstone is consistent with a cut-off channel in a braided stream deposit (Fluvial). Suggests diverse, cool climate rainforest dominated by temperate and tropic-montane rainforest taxa.	60	(Hill and Carpenter, 1991; Hill, 1989, 1991; Jordan and Macphail, 2003)
H	Hasties (H)	TAS	Mid-Late Eocene (40-34Ma*)	Located in an abandoned tin mine approximately 7km north of the village of Pioneer.	A carbonaceous mud to slightly lignite unit, within several metres of poorly sorted quartzose gravel with poorly defined cross-bedding and well preserved fragments of wood.	A flood basin swamp. The climate was cool, seasonal, with high rainfall and cloud cover. (Fluvial)	80	(Pole, 1992)
LLR	Little Rapid River (LRR)	TAS	Early Oligocene (34-30Ma*)	41 °09 'S, 145 °14 'E	A very fine-grained dark grey mudstone characterised by many layers of black organic material containing macrofossils. Above the organic layer is coarser grained material composed of dark grey sands, once again rich in black organic material.	A lake (Lacustrine) with a dramatic change in depositional regimes (i.e. a low energy environment like a centre of a lake grading up to fluctuating stream flow (possibly seasonal)). Vegetation is derived from inflowing creeks or from the lake edge	350	(Hill, 1994; Hill and Paull, 2003)
LR	Lea River (LR)	TAS	Early Oligocene (34-30Ma*)	41" 30'S, 145" 39'E; eroded riverside cliff	The sediments represent three different phases of riverine deposition, with planar-bedded, fine-grained muds	temperate ever-wet conditions, riverine (Fluvial) deposition	670	(Hill, 1994; Hill and Paull, 2003)
GF	Golden Fleece (GF)	TAS	Late Oligocene - Early Miocene (28-22Ma*)	41° 18'S 148° 10'E	Interbedded coarse sandstones with ripple laminated with cross bedded siltstones. Siltstones becoming pallid down section before becoming dark grey and organic-rich.	Closed canopy cool temperate rainforest. Possibly represents the litter from exposed forest edges. (Fluvial)	40	(Conran and Christophel, 2004; Macphail, 2012)
P	Pioneer (P)	TAS	Oligocene - Early Miocene (28-16Ma**)	41" 20'S; 147" 80'E; Pioneer tin mine	6m thick, of cross bedded and nearly horizontally stratified gravels, large scale trough cross-bedded granules with very coarse sands and minor gravels, planar cross-bedded sands, and units rich organic matter with associated sandy clays	Alluvial fan	0	(Hill and Macphail, 1983)
M	Monpealyata (M)	TAS	Late Oligocene - Early Miocene (26-22Ma**)	42"04'S, 146"40'E	It is a small siltstone lens, approximately 15 m long and about 1.5 m thick.	Represents the litter from exposed forest edges, remains of a high altitude lake. This forest edge habitat at high altitudes may represent the environment in which the alpine/subalpine flora evolved. (sub-alpine Lacustrine)	920	(Greenwood, 1994; Hill, 1988; Hill and Paull, 2003; Macphail et al., 1994; Macphail et al., 1991)
K	Kiandra (K)	NSW	Miocene (22-18Ma**)	35"52'S; 148"29'E; Homeward Bound Diggings, New Chum Hill	Rapidly deposited, poorly-sorted arenaceous coarse sands grading upwards, argillaceous clay facies consisting of well-sorted, very fine, laminated sediments which are carbonaceous.	Long periods of lake stagnation (Lacustrine) Warmer (possibly lower altitude) conditions	1375-1600	(Paull and Hill, 2003)

Table 2: Summary of literature for the sites used in this study, including species found (both angiosperm and gymnosperm), deposit description, elevation above seawater, depositional environment. * indicates that the age of the site was identified through palynology means. ** indicates that the age of the site was identified through isotopic (K-Ar) dates for an overlying basalt or igneous event. The symbols for each of the sites correspond to the dispersed cuticles figures.

Table 3: Summary of literature for the sites used in this study, regarding the flora of the. Notes: percent of gymnosperm taxa, uses the palynology of microfossils not macrofossil,, reproduced from Macphail, (2007). The symbols and colour for each of the sites correspond to the dispersed cuticle sites for all figures

Symbol	Name	Place	Dominant Vegetation Taxa	Estimated Forest Type	Estimated Climate	Percentage of Gymnosperm taxa	Elevation (m)	References
RP	Regatta Point	TAS	Angiosperms: Eucryphia, , Banksia, Quintinia, Gymnosperms: Dacrycarpus, Libocedrus Acmopyle, Bowenia, Pterostoma	MVF	±Wet Upper Microthermal	~20 %	10	(Pole, 1992; Pole and Macphail, 1996; Wells and Hill, 1989)
NC	Nelly Creek	SA	Angiosperms: Casuarinaceae (Gymnostoma), Lauraceae, Myrtaceae, Proteaceae and Sterculiaceae (Brachychiton). Gymnosperms: Araucariaceae, Podocarpaceae, Agathis/Wollemia	ScF/MVF	Moderate ±Dry Upper Mesothermal (~18-21)	35-45 % High gymnosperm abundance	1100	(Hill and Christophel, 2001)
A	Anglesea	VIC	Angiosperms: Families Gymnostoma, Ebenaceae, Lauraceae, Mertaceae, Nothofagus,; Gymnosperms: Pterostoma Bowenia Dacrycarpus.	SNVF	Very Wet - Wet Mid Mesothermal	20-30 % Variable in lenses (can be gymnosperm dominate or angiosperm dominant)	238	(Christophel et al., 1987; Macphail, 2007; Macphail et al., 1994)
GG	Golden Grove	SA	Angiosperms: Elaeocarpaceae, Lauraceae, Myrtaceae, Proteaceae, Sterculiaceae, Paracordyline; Gymnosperm: Podocarpaceae family, and a fern in the Lygodiaceae.	CNVF	±Wet Upper Mesothermal (~18)	20-30 %	120	(Hill, 1994; Macphail, 1994)
LA	Loch Aber	TAS	Angiosperms: Eucryphia and Banksieaephyllum; Gymnosperms: 3 species of Dacrycarpus Acmopyle, single species of Phyllocladus Willungia, Araucaria	MFF/SNVF	Very Wet - Wet Seasonal Microthermal	-	60	(Hill and Carpenter, 1991; Hill, 1989, 1991; Jordan and Macphail, 2003)
H	Hasties	TAS	Angiosperms: two species of Laurophyllum, Nothofagus; Casuarinaceae; Proteaceae, Myrtaceae; Gymnosperms: 12 species of Podocarpaceae, two species of Dacrycarpus, two species of Prumnopitys, Smithtonia jonesii, and three unknown species.	MFF/SNVF	Very Wet - Wet Seasonal Microthermal	-	80	(Pole, 1992)
LLR	Little Rapid River	TAS	Angiosperms: Cyatheacidites, 4 species of Nothofagus, Proteaceae, Casuarinaceae and Eucryphia. Gymnosperms: Fitzroya, Dacrycarpus, Microstrobos, Lagarostrobos, Mesibovia, 3 species of Podocarpaceae	MVF	Very Wet - Wet Mid Microthermal	70-90 %	350	(Hill, 1994; Hill and Paull, 2003)
LR	Lea River	TAS	Angiosperms: 4 species of Nothofagus, Proteaceae, Casuarinacea and Eucryphia. Gymnosperms: Araucariaceae, at least five Podocarpaceae species and four cupressaceous genera, Athrotaxis, Austrocedrus, Libocedrus, and Fitzroya.	MVF	Very Wet - Wet Upper Microthermal (~12)	49-70 %	800	(Hill, 1994; Hill and Paull, 2003)
GF	Golden Fleece	TAS	Angiosperms: Nothofagidites, Myrtaceidites verrucosus f. rhodamnoides and Sapotaceoidaepollenites rotundus, Beauprea, Strasburgeria,and Quintinia; Gymnosperms: 4 Podocarps and Araucaria.	-	-	-	40	(Conran and Christophel, 2004; Macphail, 2012)
P	Pioneer	TAS	Angiosperm: Nothofagus, Quintinia, Cupaniae, Ilex, Cunoniaceae, Mrtaceae, Proteacea and Winteraceae; Gymnosperms: Athrotaxis, Phyllocladus, Podocarpus, Dacrydium, Dacrycarpus Fitzroya and Araucariaceae	MFF/SNVF	±Wet Upper Mesothermal	~35 %	0	(Hill and Macphail, 1983)
M	Monpealyata	TAS	Angiosperms: Nothofagus, Microstrobos Isoetes, and Proteaceae; Gymnosperms: 2 species of Phyllocladus, 3 species of Dacrycarpus, Fitzroya, Araucaria,	NMF/T	Very Wet - Wet Lower Microthermal (~7.5)	57-88 %	920	(Greenwood, 1994; Hill, 1988; Hill and Paull, 2003; Macphail et al., 1994; Macphail et al., 1991)
K	Kiandra	NSW	Angiosperms: Podocarpaceae (Phyllocladus, Podocarpus), Lauraceae and Myrtaceae; Gymnosperms: Dacrycarpus	-	±Wet Lower Mesothermal	25-30 %	1375-1600	(Paull and Hill, 2003)

The age ranges for the dispersed cuticle is shown in figure 3. Three of the sites, Golden Fleece, Pioneer and Monpealyata, potentially span into two epochs, from the Oligocene to the Miocene. The Eocene span is from 56Ma through to 38Ma, the Oligocene spans 38Ma to 22Ma, and the Miocene covering 22Ma to 5Ma. Regatta point spans the smallest age range during the early Eocene, with Hasties also showing a small age range during the Mid Eocene (Macphail, 2007). Pioneer and Nelly Creek show the largest age ranges, Oligocene to Miocene, and Mid Eocene, respectfully.

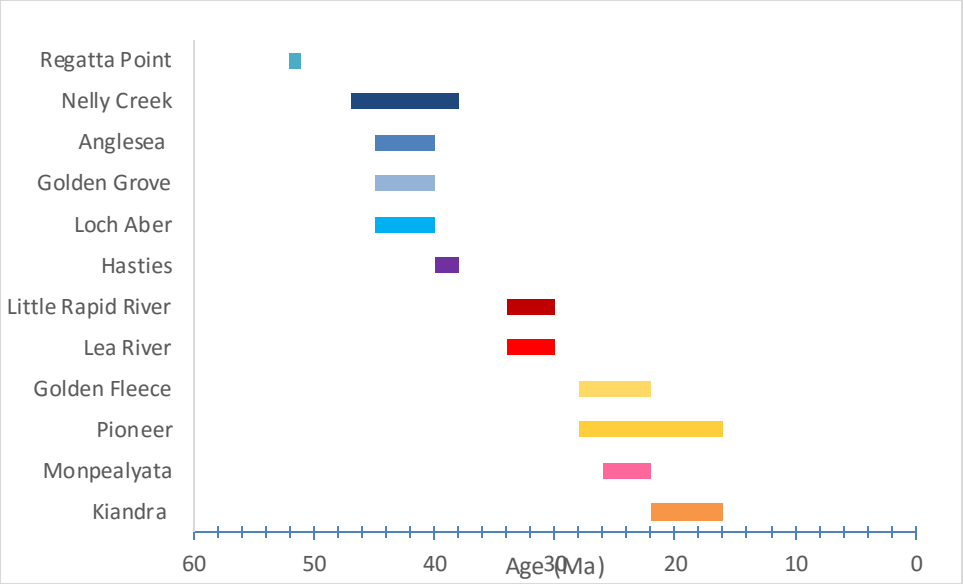

Figure 4: The fossil sites, from oldest to youngest, the colours correspond to the sites list both here and in tables 2 and 3. Ages produced from either radiometric dating from overlying basalts, or through biostratigraphy (palynology) dating.

METHODS

Sampling method

Hill and colleges treated the rock samples collected at each of the sites with hydrogen peroxide, separated the leaves from the organic and sediment material. After separated (Hill and colleges, 1983^{+}) used the whole leaves for various projects including: morphologically assessments, evaluating taxa present, and dispersed cuticle evaluation, etc. The remaining leaf samples were stored in large glass jars, with deionised water and others with ethanol. Bulk cuticle material for this study taken from the dispersed cuticle jars for each site and divided into two subsamples. The jars were mixed, with spoons, then sampled directly to get a representative sample of the cuticle. Samples were then placed, individually, in chromic (III) acid for approximately 50-60 hrs, based upon the colouration of the cuticle fragments (black cuticles require longer and clear to pale yellow suggested finished) then finally rinsed five times with deionised water and fine tipped paint brushes were used to remove adhering dirt and tissues.

Leaf fragments was visually examined under microscope to determine whether they are angiosperms or gymnosperms, as well as taxa if possible. This was done using the following criteria: angiosperms have stomates arranged in areoles, complex veining networks, random cell arrangement, gymnosperms have linear arrangements of cells, veins as well as their stomates. Individual angiosperm leaf fragments were placed in Eppendorf tubes, and dried at 40°C for approximately 18 hours. Fragments were homogenised by grinding in the tubes using a glass-stirring rod, mortar and pestle. Approximately 1-1.5mg weighed into tin boats for $\delta^{13}C$ analysis.

The dispersed cuticle samples were prepared from the same jars as the individual leaf samples. Cuticle was separated from the larger fragments, placed in Eppendorf tubes as a composite of the population; dried at 40°C. Cuticle was ground in the tubes using a glass-stirring rod and approximately 1-1.5mg weighed in to tin boats.

Samples on inspection are covered in contaminants, such as soils, spores, and leaf tissue. A test was run to investigate if the presence of contaminants result in isotopic differences. The samples involved were Eocene aged leaves from Nelly Creek. South Australia. Large angiosperm leaves were first treated with water, split in half and placed in vials and labelled. One half had the remains of the sediments and tissue on, the other were treated with chromic acid for approximately 48hrs at room temperature; paint brushes and water to remove excess acid and soils. Then both sets prepared, through grinding and weighted into the tin boats, for $\delta^{13}C$ isotopic analysis.

Isotopic analysis

The samples were analysed in the University of Adelaide using a EuraVector EuroEa Elemental Analyser in line with a Nu Instrument, No Horizon series continuous flow mass spectrometer. Samples were measured with 10% duplicates, approximately 10 for the individual, and 10 for the dispersed cuticle, corrected for sample size and instrumental drift, reported relative to Vienna Pee Dee Belemnite (VPDB (Coplen et al., 2006). The errors were evaluated using in-house standards, glycine, glutamic acid. Instrument precision was 0.05‰.

Carbon discrimination

The carbon isotope fractionation between leaf carbon and atmospheric CO_2, was computed as:

$$\Delta = \left(\delta C^{13}{}_{CO_2} - \delta C^{13}{}_{leaf}\right)/(1 - \delta C^{13}{}_{leaf}/1000) \quad (1)$$

Fractionation during carbon fixation reflects fractionation as CO_2 diffuses across plant stomata during enzymatic fixation of sugars (Condon et al., 1990; Graham et al., 2014). Where $\delta^{13}C$ is the ratio of $^{13}C/^{12}C$ in the sample, compared to the ratio in the international standard, a Cretaceous belemnite formation at Peedee in South Carolina, USA (Cernusak et al., 2008***)***. The $\delta^{13}C_{CO2}$ is the carbon isotope composition of the atmosphere, and $\delta^{13}C_{Leaf}$ is the bulk carbon isotopic composition measured in plant tissue.

A mixing model was used to assess the gymnosperm and angiosperm components of the Δ_{leaf} of the dispersed cuticle, this was calculated based on the following equation:

$$\Delta_{Final} = \Delta_{Gymnosperm} \times \%_{Gymnosperm} + \Delta_{Angioserm} \times (1 - \%_{Gymnosperm}) \quad (2)$$

Where Δ_{Final} is the mean dispersed cuticle Δ value, $\Delta_{Gymnosperm}$, $\Delta_{Angiosperm}$ is the mean Δ value for the expressed component, and $\%_{Gymnosperm}$ is the percentage of gymnosperm taxa of a given site, expressed in *table 3*. The equation was solved for $\%_{Gymnosperm}$ to give the influcene of the gymnosperms on the dispersed cuticle value. This was used for the two individual leaf sites, Anglesea and Kiandra. Anglesea had the Δ values, for dispersed cuticle, gymnosperms and angiosperms. Whereas, Kiandra only has the Δ values, for dispersed cuticle, angiosperm, with the gymnosperm being corrected assuming the gymnosperm Δ value followed the 2% enrichment of $\delta^{13}C$, similar to Anglesea and modern day forests.

Atmospheric CO_2 $\delta^{13}C$ reconstructions

There are two Cenozoic reconstructions of $\delta^{13}C_{CO2}$ established from a meta-analysis of planktonic and benthic foraminiferal $\delta^{13}C$ and $\delta^{18}O$ records, provided by Tipple et al (2010). Tipple et al (2010) concluded that due to the lower temporal resolution and high isotopic variability in modern species, planktonic foraminifera records have a limited function in reconstructing $\delta^{13}C_{CO2}$ across extended geologic timescales. The high-resolution benthic foraminifera $\delta^{13}C$ and $\delta^{18}O$ records constrained $\delta^{13}C_{CO2}$ values through systematically treating the environmental, diagenetic and species-specific effects of these records (Tipple et al., 2010). As such this study will be using the benthic foraminifera as the atmospheric $\delta^{13}C_{CO2}$ reconstruction.

RESULTS

Individual leaf measurements

The calculated Δ_{leaf} values for the individual leaf fragments, from Anglesea and Kiandra, are shown in figure 6. Anglesea shows a large range of values across the three sample types: angiosperms, gymnosperms and dispersed cuticle. The angiosperms, from Anglesea, show large Δ_{leaf} values, compared to the other types, ranging from 31.5±0.52‰ to 25.1±0.52‰. Whereas, the gymnosperms have lower Δ_{leaf} values, ranging from 29.4±0.52‰ to 22.8±0.52‰, with dispersed cuticle having a smaller range of values 26.76±0.52‰ to 25.76±0.52‰. This suggests that the angiosperms are more depleted in $\delta^{13}C$ resulting in the large Δ_{leaf}, than the gymnosperms.

Kiandra also shows a large range across the two sample types, angiosperm and dispersed cuticle. The angiosperms again have larger Δ_{leaf} values, with the dispersed

cuticle lower in Δ_{leaf}. The angiosperm values range from 30.36 ± 0.51‰ to 23.85 ± 0.51‰, with the dispersed cuticle ranging from 26.0 ± 0.51‰ to 26.8 ± 0.51‰ see figure 6. Both floras both have large ranges across the angiosperms, gymnosperms and dispersed cuticle, with the larger values attributed to the angiosperms and the lowest to the gymnosperms or dispersed cuticle, indicating an isotopic gradient.

The mean Δ_{leaf} values, from the two fossil sites, have a range of values associated, caused by the $\delta^{13}C_{CO2}$ of the age span. The mean values of the fossil sites shown are significantly more depleted in ^{13}C, resulting in the larger Δ_{leaf} values. Anglesea has a larger mean value at 28.01 ± 0.52‰, with Kiandra having a lower value of 27.45 ± 0.51‰, with sample sizes of 48 and 53 for the two sites respectively. Both means are from the angiosperms, as the dispersed cuticle and gymnosperm sample sizes are smaller. The mean of the 20-sampled gymnosperm from Anglesea is 25.75 ± 0.51‰, which is considerably smaller than the angiosperms.

The mean Δ_{leaf} values of the three different leaf types, for Anglesea show statistical variation between the sites summarised *table 4*. The isotopic Anglesea observations are similar to that of the modern day observation of angiosperm-gymnosperms, this being that gymnosperms differ from angiosperms by 1-2‰ (Diefendorf et al., 2010). The dispersed cuticle from Anglesea also shows enriched values, statistically comparable to the gymnosperms than the angiosperms (see *table 4*). Both the gymnosperms and the dispersed cuticle from Anglesea have larger Δ_{leaf} values than that of the modern day forests, seasonal and tropical. The reconstructed Δ_{leaf} values for the gymnosperms and the dispersed cuticle being 25.75± 0.52‰ and 26.18 ± 0.52‰, compared to the largest Δ

$_{leaf}$ values of the modern day, seasonal and tropical forests of 21.2‰ and 21.8‰ respectfully.

Table 5: showing the t-test results between the different samples. Significance is at P<0.05. Anglesea Angiosperms (AA), Anglesea Gymnosperms (AG), Anglesea Dispersed Cuticle (AC), Kiandra Angiosperms (KA), Kiandra Dispersed Cuticle (KC), Nelly Creek Angiosperms (NCA) percentage of gymnosperm taxa (% G), dispersed cuticle sites (DC)

T TESTS	P VALUE	SIGNFICANT
AA - AG	5.12E-05	YES
AA - AC	3.01E-06	YES
AG - AC	0.835381	NO
KA – KC	0.000334	YES
AC – KC	0.422399	NO
AA – KA	0.005962	YES
AA - NCA	1.85E-10	YES
KA - NCA	9.22E-08	YES
%G - DC	6.31E-06	YES

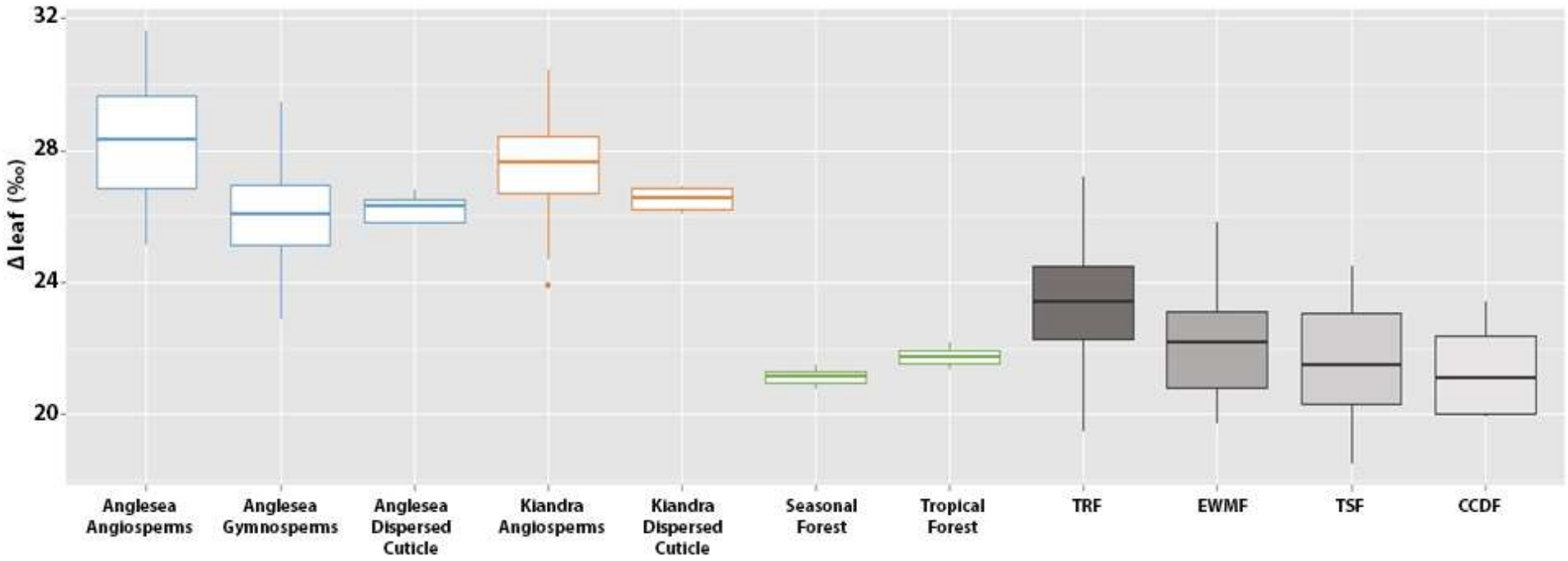

Figure 6: Both individual leaf Δ_{leaf} for angiosperms, gymnosperms as well as $\Delta_{leaf\ for}$ dispersed cuticle, Anglesea and Kiandra. The Anglesea angiosperms and gymnosperms reflect the offsets shown in modern day angiosperms and gymnosperms shown by (Diefendorf et al., 2010). Dispersed cuticle samples, for Anglesea and Kiandra, have a sample size of 10, with Anglesea and Kiandra angiosperms have samples ~50 each, with Anglesea gymnosperms having a size of 20. The fossil sites represent the mean $\delta 13C_{leaf}$ value for the benthic foraminifera atmospheric proxies. The mean Δ_{leaf} values of two modern day forests, seasonal and tropical, reproduced from Graham et al (2014). These two forests represent the different canopy structures; the seasonal characterizes the open canopy forests, with the tropical the closed forest, reproduced from the Graham et al (2014) model for the sample size of 50. Diefendorf et al (2010) forest biome types are also include, which range from tropical rainforest (TRF), evergreen warm moist forest (EWMF), tropical seasonal forest (TSF), and cool cold deciduous forest (CCDF). These forests show possible modern day analogues, for the different forest types.

The range of $\delta^{13}C_{leaf}$ values for two sites, Anglesea, and Kiandra, shown in figure 7, with the results from Graham et al. (2014) study for the two modern day forests for comparison. The seasonal forest is less variable comparatively to the tropical forest. The 50% of the data, for the seasonal forest, falls between the values of ~4.5 to 5.5, with some overlap with the tropical forests, which 50% falling between the values ~5 to 9.5. However, these modern forests are the result of a bootstrapping model, produced from Graham et al. (2014), whereas the fossil sites have not been modelled. The fossil sites have a relative consistent range of ~6, with Anglesea angiosperms with the value of 6.1‰, Anglesea gymnosperms of 6.2‰ and Kiandra 6.2‰. The gymnosperms have a large range of values for a sample size of ~20, compared to Anglesea angiosperms and the Kiandra angiosperms both having a sample size of ~50. The high ranges is comparable to the closed canopied tropical forest of the modern day.

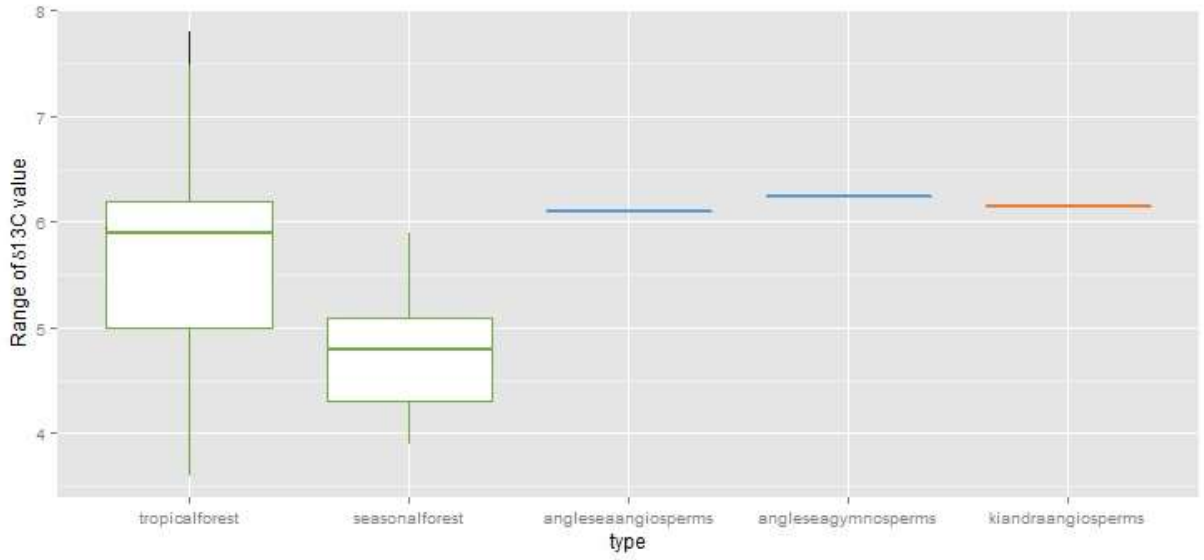

Figure 7: Isotopic ranges, the modern day forests, tropical and seasonal, (Graham et al, 2014) returned by 2000 iterations of the resampling model for the discrete sample size of 50, expressed as a boxplot, for maximum and minimum values, generated by the Graham et al (2014) model. The fossil sites have not been resampled, and are expressed as mean lines. Anglesea gymnosperms have a sample size of 20, the other sample, types have sample sizes of 50. Figure is arranged from the recent sites, the seasonal and tropical forests, to the Eocene, Anglesea, and finally the Miocene site, Kiandra.

Dispersed cuticle measurements

The Δ_{leaf} mean values for the dispersed cuticle sites, described in table 2, compared with their percentage of gymnosperm taxa observed in the sites, see figure 8. Each of the fossil sites shows varying degrees of gymnosperm abundance, with some of the sites being completely dominated by gymnosperms, such as Loch Aber (70% gymnosperm) and Monpealyata (72.5 ± 15.5 %). Other sites such as Anglesea have varying dominance depending on the location in the stratigraphic section. The Δ_{leaf} values suggests that there is a relationship between Δ and percentage of gymnosperm taxa present at each site. A Linear regression reveals that there is a relatively strong relationship as there is an R^2 value of 0.71, with a corresponding p value of 6.31E-06. A test to see the influence the gymnosperms had on the values were calculated from the mixing equation, equation 2. This showed that the gymnosperms contributed 82.4% for Anglesea and 50.5% for Kiandra, which are inconsistent with the value shown in table 3.

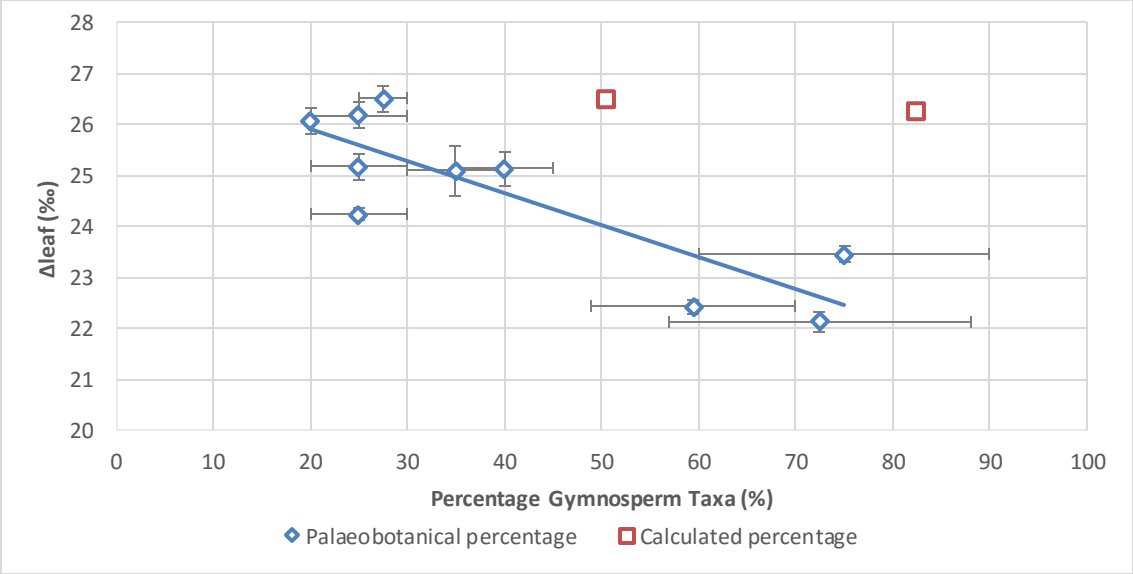

Figure 8: The percentage of gymnosperm taxa of each site, shown in table 3, observed in the literature compared to the corresponding Δ_{leaf} values. The colours indicate site, also shown in tables 2 and 3. The symbols indicate whether the values recorded from palaeobotanical studies or calculated using equation 2. The squares symbols are for the two fossil sites: Anglesea and Kiandra, which had the gymnosperm abundances calculated; the diamonds are the palaeobotanical.

The dispersed cuticle measurements show large variations in the mean Δ_{leaf} values, see figure 9. The Eocene sites, Regatta Point, Nelly Creek, Anglesea, Golden Grove, Loch Aber, and Hasties, show a large array of Δ_{leaf} values. Anglesea, Golden Fleece and Nelly Creek, have more enriched Δ_{leaf} values, 26.26± 0.25‰, 25.18± 0.25‰, 25.13± 0.35‰ respectively, than the other Eocene sites, with Loch Aber showing a smaller Δ_{leaf} value of 22.52± 0.25‰. Little Rapid River, from the Oligocene, shows a lower Δ_{leaf} of 23.46± 0.15‰, however, it is higher than the Δ_{leaf} value of 22.42± 0.15‰ from Lea River, which is also an Oligocene site and located down river from the Little Rapid River. The Oligocene-Miocene sites, Golden Fleece, Pioneer and Monpealyata, show differing Δ_{leaf} values. Golden Fleece and Pioneer, have high Δ_{leaf} values comparable to the high value Eocene sites, with values of 26.06 ± 0.25‰ and 25.10± 0.50‰ respectively. Whereas Monpeelyata is more depleted in Δ_{leaf}. The Miocene site, Kiandra, has a larger enrichment of Δ_{leaf}, than the Eocene sites, with a value of 26.51 ± 0.25‰, compared to 26.26± 0.25‰ from Anglesea.

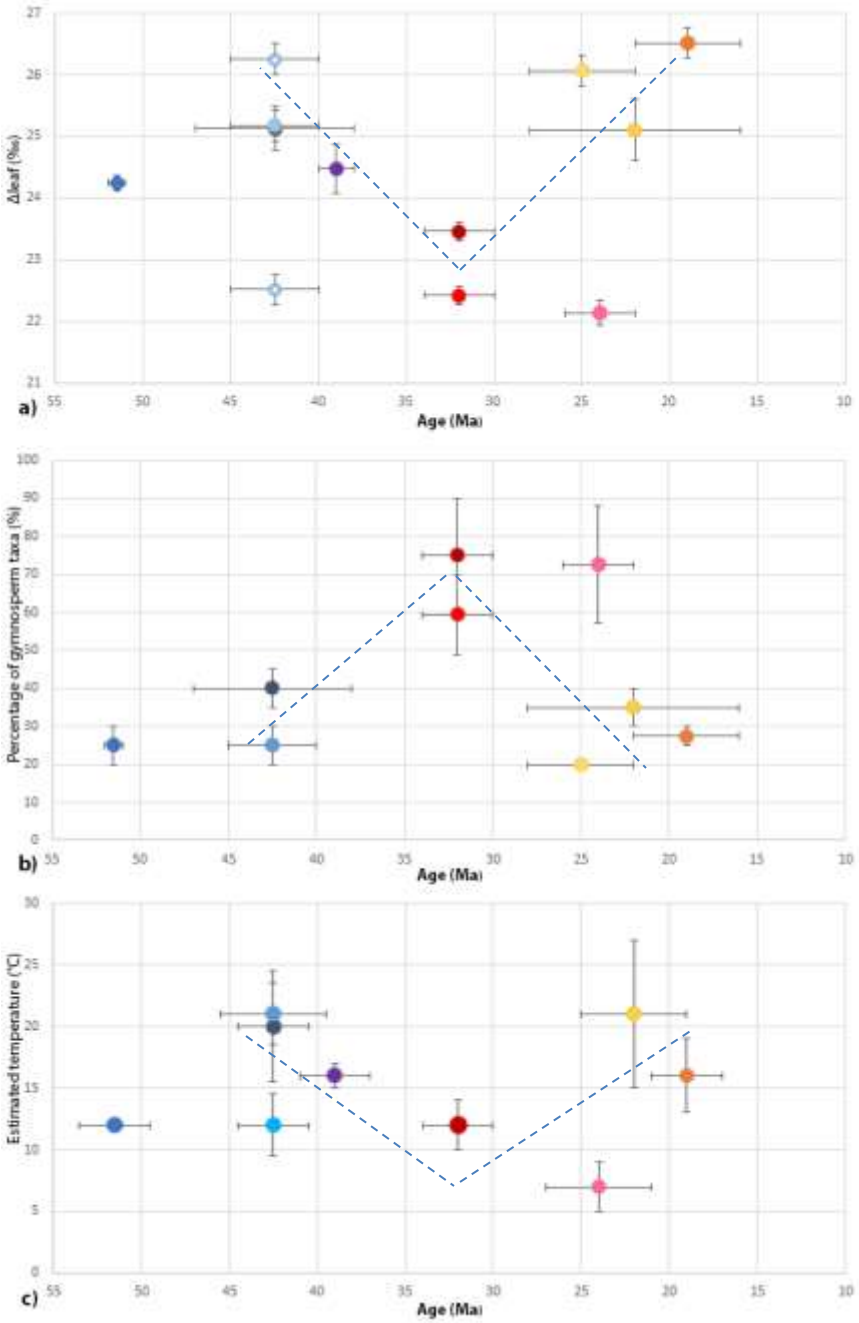

Figure 9: The fossil sites span 52Ma (Eocene) to approximately 16Ma (Miocene), with each of the colours representing the different sites, shown in table 2 and 3. The dashed line indicates an inferred trend the data follows. (a) Shows the mean dispersed cuticle Δ_{leaf} values across the ages span. The benthic foraminifera was used for the atmospheric reconstruction in the Δ_{leaf} values, range of the age indicates the possible ages of each site, whereas the Δ_{leaf} range shows the atmospheric variability in the reconstruction across the age. (b) Shows the percentage of gymnosperm taxa change also shown in table 3. The percentages of gymnosperms taxa, reproduced from (Macphail, 2007), is hard to distinguish as some fossil sites have very low abundances of gymnosperms. (c) Shows the estimated temperature from the dispersed cuticle sites, described in tables 1 and 3. These estimates are placed into categories, from microthermal to megathermal (for definitions see table 1), and represent the error in the temperature. Most of the dispersed cuticle sites have an estimated climate associated with them, see table 3 (Macphail, 2007)

Acidification test

The samples run for the individual leaf and the dispersed cuticle analyses were first treated with chromic (III) acid (chromium trioxide) before being analysed for bulk carbon. This was the result the untreated leaves showing a shift in $\delta^{13}C$, see figure 10. The untreated leaves were visually observed to be more negative, suggesting that the contamination is organic matter as it is more depleted in $\delta^{13}C$, than cuticular measurements. There was a visual shift as the untreated leaves were more negative than the treated leaves, suggesting organic matter contamination.

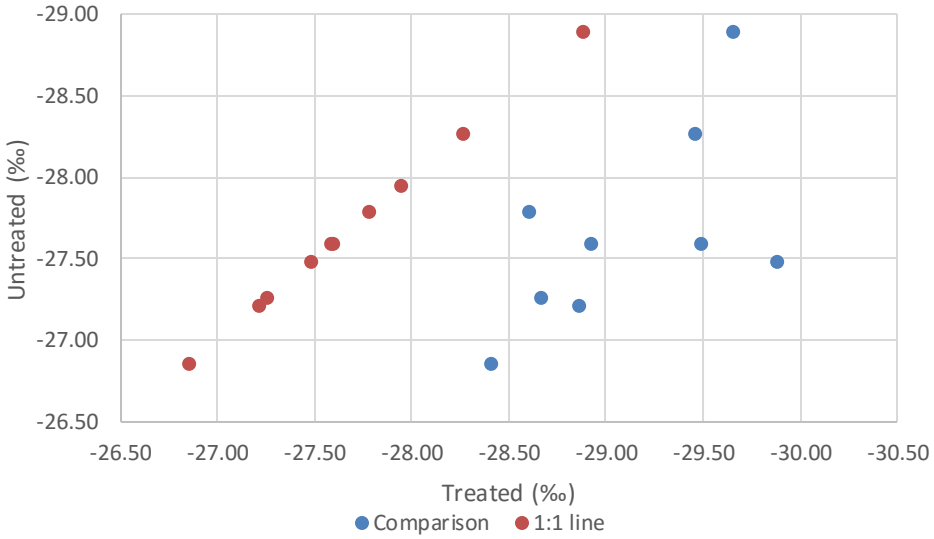

Figure 10: Comparison of acidic treated and untreated *Myrtaceae* leaves from Eocene leaves in Nelly Creek. The blue indicates treated, and the orange represents the untreated. Each sample represents one lead that has been measured for bulk carbon twice, once treated with acid and once without, this was to remove any morphological / taxa bias, used to assess possible contamination in fossil leaves.

DISCUSSION

Two methods were used to reconstruct the canopy closure of south-eastern Australia during the Cenozoic. An individual leaf analysis was used to assess whether the stable isotope ratios reflect canopy closure from the Eocene to the Miocene using modern North and Central American analogues. The second was assessing whether dispersed cuticle can be used as a cheaper and less destructive method to determine canopy closure. As such, would dispersed cuticle samples reflect the average angiosperm $\delta^{13}C$ values, and if not what does it reflect?

Individual leaf

Modern day comparison between angiosperms and gymnosperms presents a difference in mean Δ_{leaf} values. Gymnosperms Δ_{leaf} values are lower by up to 2‰ than for angiosperms (Diefendorf et al., 2010). A comparison between the Anglesea angiosperms and gymnosperms, seen in figure 6, show that there is a 2‰ difference between them, suggesting that we are dealing with original carbon isotopic signatures. Alternatively if the samples from the gymnosperms and angiosperms showed no difference in the Δ_{leaf}, i.e. if they were the same, they could be considered to be not original. There are several causes for this, including altered during diagenesis, contaminated, overprinted from when the plant grew. As such there would be no canopy effect preserved. Given that the angiosperms and gymnosperms from Anglesea have the 2‰ difference, the Δ_{leaf} values suggest original carbon isotopic signatures, and possibly the preservation of the 'canopy effect'.

The range of $\delta^{13}C$ values from the Anglesea angiosperms and gymnosperm, seen in figure 7, show similar values, that is 6.104‰, and 6.234‰ respectively. This suggests that both samples types span the canopy. However, there are two scenarios that fit this observed gradient. The first

is a vertical isotopic gradient, called the 'canopy effect'. The second is a horizontal gradient, given by a margin of the forest that is more open than that of the forest, this could be for a body of water or a completely different environment, and increases with more depleted $\delta^{13}C$ values away from the margin. Given the similarity of the isotopic range for the angiosperms and gymnosperms, the data implies that the two sample types span the same isotopic environments as well as suggesting original carbon isotopic signatures, beause the isotopic variations are preserved.

Modern day forests with different canopy closures show different isotopic mean and range signatures. Graham et al., (2014) study in the closed tropical forest and the open seasonal forest showed different isotopic values. The tropical forest showed a mean Δ_{leaf} value of 22.59‰ with a mean range of 5.9‰, and the seasonal forest 21.08‰ and mean range of 4.8‰. This indicates that the closed forests are more depleted in $\delta^{13}C$ and have larger ranges than the open forests (Graham et al., 2014). Given that the isotopic signatures in the Australia fossil flora are persevered, and the closure of a forest can be assessed using the isotopic mean and range of values, the method should be able to reconstruct canopy closure.

Using both the isotopic mean and range of values the closure of two sites: Anglesea (A) and Kiandra (K), showed similarity, even though they are separated by ~20Ma. The angiosperms from the Eocene site, Anglesea, showed a high mean Δ_{leaf} value of 28.01 ± 0.52‰, with a range of 6.104‰, seen in figures 6, 7. The angiosperms from the Miocene site, Kiandra, showed similarly high mean value of 27.45 ± 0.51‰, with a range 6.2‰, seen in figures 6, and 7. Both

sites have depleted values with large ranges, compared with the two modern day closure forests, i.e. the tropical and seasonal forests.

The modern canopy analogs, the tropical and seasonal forests, differ substantially to the paleo sites. The modern forests, have a collective range of ≈20.9‰ to ≈22.1‰ whereas the fossil sites have a collective range of ≈27.0‰ to ≈28.1‰, giving a difference of approximately 5‰. There a reason for this offset is most likely associated with tissue types. In this study the samples were treated in chromic (III) acid, between 50-60 hrs, before being homogenized to leave only the cuticle; whereas Graham et al., (2014), only homogenized the samples, leaving the different tissues in the leaf. As such, the cuticle tends to be more 13C-depleted compared bulk leaf samples, as there is an isotopic enrichment of plant cellulose relative lignin (A. Hobbie and Werner, 2004; Benner et al., 1987). This leads to a difference in Δ_{leaf} values as seen in figure 6.

Fricke (2007) and Grein et al., (2010) showed that it is possible to reconstruct canopy structure in the fossil record. Fricke (2007) results from Castle Rock, early Cenozoic (64.1Ma), show that the $\delta^{13}C$ values were relatively depleted, the $\delta^{13}C$ ranged from -28.8‰ to -24.8 ‰. This is more enriched than the results from Anglesea and Kiandra, which ranged from -33.6‰ to -27.5‰ and -33.2‰ to -26.2‰ respectively. In saying this, Fricke (2007) suggested that the canopy might not be represented well in the fossil leaf litter. The results from Grein et al ., (2010) is more comparable to Anglesea and Kiandra, with the $\delta^{13}C$ of the fossil cuticles from *Lauraceae* and *Myrtaceae* varies from –30 ‰ to –27 ‰ and–29 ‰ to –26 ‰ respectively. This suggests that the forests of the Messel pit in Germany, Early Cenozoic are more closed, similar to those found in Anglesea and Kiandra.

Angiosperms from both Anglesea and Kiandra have large mean values and large ranges indicating their canopy closure, as well as possible biomes. Given their large mean values as the study conducted by Diefendorf et al., (2010), the forest type the fossil sites are isotopically analogous to are the TRF, tropical rainforests. However, previous morphological studies on these sites indicate a different environment that these plants grew in. Anglesea is suggested to have a more moderate mesothermal (*temperate*) environment with very high rainfall, (Christophel et al., 1987; Macphail, 2007). Kiandra also had similar conditions with lower mesothermal (*temperate*) with very high rainfall (Macphail, 2007; Paull and Hill, 2003). The morphological studies greatly differ to the modern forest analogs. The Morphological studies predict higher humidity with lower temperatures, than the forest analogues high temperature, high humidity similar totropical rainforests.

Morphological observations from the Kiandra fossils provide information about the climate and environment in which these plants evolved. The observations include drip tips, and on the cuticles, Florin rings, papillose cuticular ornamentation and cuticular striations as well as the absence of these occurrences (Tarran, 2013). Most of these observation are adaptions for the removal of water quickly and efficiently (Malhado et al., 2012; Tarran, 2013) indicting that the environment, in which they evolved, was ever-wet (Hill, 1988; Tarran, 2013). The removal of water is essential as these adpations prevent the invasion of the stomates from fungal hyphae (Tarran, 2013). Some of these observations have been made in Anglesea, such as drip tips, fungal hyphae, florin rings. These seem to be consistent with the isotopic observations of the individual leaf results.

Dispersed cuticle

Dispersed cuticle is a new possible way to rapidly assess the closure of an ancient forest. These fossil plant fragments are a possible source that encompasses the entire ecosystem at the time of deposition. Similar to the average sample of many individual leaves, the dispersed cuticle samples preserve the average mean isotopic signatures for the ecosystem, which will allow quick assessment of the canopy cover. It is a considerably cheaper way to look at the forest ecosystem, and is less damaging to the fossil collect than the individual leaf method.

The dispersed cuticle samples the entire population, including the different flora taxa present in the forests. The dispersed cuticle shows a large influence from the gymnosperm taxa. The individual leaf results for Anglesea, figure 6, show that the gymnosperms have a larger proportion in the dispersed cuticle than the angiosperms. The mean Δ_{leaf} values for the angiosperms, gymnosperms and dispersed cuticle are as follows, 28.18 ± 1.80‰, 25.86 ± 1.72‰ and 26.26 ± 0.25‰. The Miocene site, Kiandra, also showed that the angiosperms are not as influential as the gymnosperms as the dispersed cuticle does not equal the mean angiosperm values. These are 27.53 ± 1.32‰, for the angiosperms, and 26.52± 0.25‰, for the dispersed cuticle samples. This shows that there may be a preservation bias in the fossil record for the dispersed cuticle. However, the dispersed cuticle may still show the canopy effect on a broader scale.

This gymnosperm bias, in the dispersed cuticle, shown in the individual leaf samples also shown in the dispersed cuticle samples. The estimated percentage of taxa shown in table 2 shows that there are sites where gymnosperms are the dominate taxa in the deposit. These sites show significant contribution of the cuticle are from gymnosperms, as seen in figure 6 and 8. Using

the mixing equation (2), to calculate the gymnosperm contribution, we see that Anglesea has a contribution of 82.8% from the gymnosperms, and Kiandra has an input of 78.0%, compared to the estimated percentage of taxa 25 ± 5% and 27.5 ± 2.5%. This support the individual leaf observations that the gymnosperm contribution to the biomass is highly influential, and is biased, possibly preservation.

The dispersed cuticle fossil sites cover a large portion of the Cenozoic, and are located around south-eastern Australia (ages and locations, see *tables 2 and 3, and map 1*). They have differing taxa abundances as well as diverse geographic and climatic environments, see *table 2*. Anglesea and Kiandra both have had the individual leaf analysis and the dispersed cuticle test. Anglesea shows that the mean angiosperm Δ_{leaf} value (28.18 ± 1.80‰) shows a larger Δ_{leaf} than the dispersed cuticle (26.26 ± 0.25‰). Similarly shown in Kiandra, as the angiosperm values are larger than the Δ_{leaf} dispersed cuticle, 27.53 ± 1.32‰, and 26.52± 0.25‰ respectively.
The dispersed cuticle samples the entire population, including the different flora taxa present in the forests. The dispersed cuticle shows a large influence from the gymnosperm taxa. The individual leaf results for Anglesea, figure 6, show that the gymnosperms have a larger proportion in the dispersed cuticle than the angiosperms. The mean Δ_{leaf} values for the angiosperms, gymnosperms and dispersed cuticle are as follows, 28.01 ± 0.52‰, 25.79 ± 0.52‰ and 26.18 ± 0.25‰. The Miocene site, Kiandra, also showed that the angiosperms are not as influential as the gymnosperms as the dispersed cuticle does not equal the mean angiosperm values. These are 27.44 ± 0.51‰, for the angiosperms, and 26.44± 0.25‰, for the dispersed cuticle samples. This shows that the dispersed cuticle represents a mixture of angiosperms and gymnosperms. Using the mixing equation (2), to calculate the gymnosperm contribution, we see

that Anglesea has a contribution of 82.8% from the gymnosperms, and Kiandra has an input of 50.5%, compared to the estimated 25 ± 5% and 27.5 ± 2.5%, based on the percentage of gymnosperm taxa. This suggests the gymnosperms contribution to the biomass is highly influential, and is potentially biased, by preferential preservation.

The dispersed cuticle fossil sites cover a large portion of the Cenozoic, and are located around south-eastern Australia (ages and locations, see tables 2 and 3). They have differing taxa abundances as well as diverse geographic and climatic environments, see *table 2*. Anglesea and Kiandra both have had the individual leaf analysis and the dispersed cuticle test. Anglesea shows that the mean angiosperm Δ_{leaf} value (28.01 ± 0.52‰) shows a larger Δ_{leaf} than the dispersed cuticle (26.18 ± 0.25‰). Similarly shown in Kiandra, as the angiosperm values are larger than the Δ_{leaf} dispersed cuticle, 27.45 ± 0.51‰, and 26.44± 0.25‰ respectively. This gymnosperm bias, in the dispersed cuticle, shown in the individual leaf samples is also shown in the dispersed cuticle samples. The estimated percentage of taxa shown in table 2 shows that there are sites where gymnosperms are the dominate taxa in the deposit. These sites show a correlation with Δ_{leaf}, as seen in figures 8 and 9.

Figure 8 suggests that there is a relationship between gymnosperms and Δ_{leaf} as the p values indicate a significance (6.31E-06) and the linear regression shows an R^2 value of 0.71. In modern day forests, gymnosperms and angiosperms differ only by 2‰, whereas the range in the dispersed cuticle values is approximately 5‰. As such, there is another cause, as the gymnosperm content alone cannot explain the 5‰, which indicated that there is a signal showing the canopy closure. Figure 9 (b) shows that the diversity of gymnosperms has changed from the

Eocene to the Miocene (Macpahil, 2007). The Eocene sites suggest that there was had low proportions of gymnosperms, with possible high localized abundances. Shown in the Anglesea, Golden Grove, and Nelly Creek , 25 ± 5%, 25 ± 5%, 40 ± 5%, and gymnosperms, respectively (Macpahil, 2007). The Oligocene shows an opposite to the Eocene, as there was an increase in gymnosperm diversity, as Little Rapid River, Lea River, and Monpealyata have large percentages of gymnosperm taxa present, 59.5 ± 10.5%, 80 ± 10%, and 72.5 ± 15.5%, respectively (Macpahil, 2007). Golden Fleece, Pioneer and Kiandra have lower gymnosperm taxa present in the sites, with ~20 %, 35% and 27.5 ± 2.5%, respectively (Macpahil, 2007). Looking at previous plant physiometry work, there have been estimates of average temperatures that the plants may have grown in, see table 2 (Macphail, 2007). The change in temperature indicated in figure 9 (c) corresponds to the change in the percentage of gymnosperm taxa in the fossil sites. During the Eocene, most of the fossil sites show largely warmer climates, i.e mesothermal, ranging from about 15°C to 25°C; these sites include Anglesea, Golden Grove, Nelly Creek, and Hasties, with the exception of Loch Aber and Regatta Point, which are locally cooler. The Oligocene shows that the fossil sites are cooler, ranging 7°C to 13°C; these sites include Little Rapid River, Lea River and Monpealyata. Kiandra and Pioneer, show that there was an increase in temperature during the Miocene, with values ranging 14°C to 25^{+}°C.

The change in gymnosperm abundance may correspond to a change in canopy structure. Biogeographic evidence shows that conifers (gymnosperms) are confined to high latitudes and elevations or nutrient-poor soils or cool climates where canopies tend to be more open (Bond, 1989; Diefendorf et al., 2010). This may suggest that Δ_{leaf} values is indicative of the change in canopy structure. Past morphological work, suggests that most of the Eocene sites are closed

canopied forests, such as Anglesea, Golden Grove Nelly Creek, Hasties, as they all have high Δ_{leaf} values, 28.01±0.25‰, 25.18±0.25‰, 25.13±0.35‰, and 24.48±0.25‰, respectively. Oligocene, Little Rapid River and Lea River, suggests that the forests are more open canopied, as they have lower Δ_{leaf}. The Oligo-Miocene sites suggest more closed canopied. However, Monpealyata is suggestive of an open canopied environment, in the literature the site is an open sub-alpine environment. The Miocene site, Kiandra, also shows a closed canopy environment, similar to the Eocene sites, with a larger Δ_{leaf} value of 26.44±0.25‰. This another plausible explanation of the observed trend seen in figure 9.

As seen by figures 8 and 9, the dispersed cuticle sampling shows a great deal of information, from changing climates, to changing ecosystems and forest types. Future work will be on eliminating variables from the dispersed cuticle samples, such as precipitation, and sample sizes, to get a clearer understanding and test to see if dispersed cuticle can be used as a proxy for canopy closure or estimating gymnosperm taxa. Identifying whether temperature is the cause for the offset Δ_{leaf}, by sampling modern day forests that have similar precipitation pattern and variable temperatures, in the southern hemisphere, as well as possible latitudinal variation in the data. The major aim will be to get a clearer understanding of abundances versus proportion of angiosperms / gymnosperms in the different sites across Australia.

CONCLUSIONS

Combining two sampling methods, dispersed cuticle and individual leaf, the canopy closure of 13 fossil sites Δ_{leaf} to produced canopy closure of fossil sites in south-eastern Australia spanning from the Eocene to the Miocene. By comparing the individual leaf and dispersed cuticle data we evaluated that the dispersed cuticle data is heavily influenced by their respective gymnosperm

content, as the dispersed cuticle sample did not equal the mean angiosperm value. We established the isotopic expression within two paleo-forests and compared them to possible modern day equivalents. The results for the individual leaf for the two paleo-forests, Anglesea and Kiandra, showed that they are both closed canopied forests, suggested by both the mean (28.01 ± 0.52‰ and 27.45± 0.51‰, respectively) and the range of isotopic values (6.01‰ and 6.25‰, respectively). The dispersed cuticle present similar results, however, not as extreme as the individual leaves, Anglesea showed a mean value of 26.18 ± 0.25‰ and Kiandra showed 26.44 ± 0.25‰, suggestive that the dispersed cuticle is influenced more by the gymnosperms. The dispersed cuticle fossil sites showed that the Eocene was more closed, the Oligocene showed a more open closure, and the Miocene had closed canopied forests, suggested by the change in temperature reflected by the gymnosperm diversity. A few sites showed exceptions; Loch Aber during the Eocene was open canopied, as well as Monpealyata, from the Miocene, showed an open forest. These findings are all consistent with morphological studies on these sites, suggesting that both methods could reconstruct the canopy closure of fossil sites.

ACKNOWLEDGMENTS

I would like to thank Kristen Nelson and Jake Andrae for their advice and invaluable help in the Stable Isotope Laboratory. Myall Tarran for his company, conversation and knowledge in the Adelaide Palaeobotany Lab, Professor Robert Hill for allowing me to work with the dispersed cuticle samples, Mark Rollog for his patience with my isotope samples and finally Professor Francesca McInerney for taking me on as a student.

REFERENCES

A. HOBBIE, E., AND WERNER, R. A., 2004, Intramolecular, compound-specific, and bulk carbon isotope patterns in C3 and C4 plants: a review and synthesis: New Phytologist, v. 161, no. 2, p. 371-385.

ARENS, N. C., HOPE JAHREN, A., AND AMUNDSON, R., 2000, Can C3 plants faithfully record the carbon isotopic composition of atmospheric carbon dioxide?: Paleobiology, v. 26, no. 1, p. 137-164.

BARBOUR, M. M., 2007, Stable oxygen isotope composition of plant tissue: a review: Functional Plant Biology, v. 34, no. 2, p. 83-94.

BEERLING, D. J., BIRKS, H. H., AND WOODWARD, F. I., 1995, Rapid late-glacial atmospheric CO2 changes reconstructed from the stomatal density record of fossil leaves: Journal of Quaternary Science, v. 10, no. 4, p. 379-384.

BENNER, R., FOGEL, M. L., SPRAGUE, E. K., AND HODSON, R. E., 1987, Depletion of 13 C in lignin and its implications for stable carbon isotope studies: Nature, v. 329, no. 6141, p. 708-710.

BETH, E., 2001, A 64.1 MILLION YEAR OLD TROPICAL RAINFOREST FROM CASTLE ROCK, COLORADO, Paleontology/Paleobotany VI: Terrestrial Paleoenvironments and Biostratigraphy, Volume 188-6: Colorado Convention Center, The Geological Society of America

BOND, W. J., 1989, The tortoise and the hare: ecology of angiosperm dominance and gymnosperm persistence: Biological Journal of the Linnean Society, v. 36, no. 3, p. 227-249.

BOYCE, C. K., AND LEE, J.-E., 2010, An exceptional role for flowering plant physiology in the expansion of tropical rainforests and biodiversity: Proceedings of the Royal Society of London B: Biological Sciences, p. rspb20100485.

BROWN, M. J., AND PARKER, G. G., 1994, Canopy light transmittance in a chronosequence of mixed-species deciduous forests: Canadian Journal of Forest Research, v. 24, no. 8, p. 1694-1703.

CERLING, T. E., HARRIS, J. M., MACFADDEN, B. J., LEAKEY, M. G., QUADE, J., EISENMANN, V., AND EHLERINGER, J. R., 1997, Global vegetation change through the Miocene/Pliocene boundary: Nature, v. 389, no. 6647, p. 153-158.

CERLING, T. E., HART, J. A., AND HART, T. B., 2004, Stable isotope ecology in the Ituri Forest: Oecologia, v. 138, no. 1, p. 5-12.

CERNUSAK, L. A., WINTER, K., ARANDA, J., AND TURNER, B. L., 2008, Conifers, angiosperm trees, and lianas: growth, whole-plant water and nitrogen use efficiency, and stable isotope composition (δ13C and δ18O) of seedlings grown in a tropical environment: Plant Physiology, v. 148, no. 1, p. 642-659.

CHRISTOPHEL, D. C., HARRIS, W. K., AND SYBER, A. K., 1987, The Eocene flora of the Anglesea Locality, Victoria: Alcheringa: An Australasian Journal of Palaeontology, v. 11, no. 4, p. 303-323.

COHEN, B. E., KNESEL, K. M., VASCONCELOS, P. M., AND SCHELLART, W. P., 2013, Tracking the Australian plate motion through the Cenozoic: Constraints from 40Ar/39Ar geochronology: Tectonics, v. 32, no. 5, p. 1371-1383.

CONDON, A., FARQUHAR, G., AND RICHARDS, R., 1990, Genotypic variation in carbon isotope discrimination and transpiration efficiency in wheat. Leaf gas exchange and whole plant studies: Functional Plant Biology, v. 17, no. 1, p. 9-22.

CONRAN, J. G., AND CHRISTOPHEL, D. C., 2004, A fossil Byblidaceae seed from eocene south Australia: International Journal of Plant Sciences, v. 165, no. 4, p. 691-694.

COPLEN, T. B., BRAND, W. A., GEHRE, M., GRÖNING, M., MEIJER, H. A., TOMAN, B., AND VERKOUTEREN, R. M., 2006, New guidelines for δ 13C measurements: Analytical Chemistry, v. 78, no. 7, p. 2439-2441.

DIEFENDORF, A. F., MUELLER, K. E., WING, S. L., KOCH, P. L., AND FREEMAN, K. H., 2010, Global patterns in leaf 13C discrimination and implications for studies of past and

future climate: Proceedings of the National Academy of Sciences, v. 107, no. 13, p. 5738-5743.

FEARY, D. A., DAVIES, P. J., PIGRAM, C. J., AND SYMONDS, P. A., 1991, Climatic evolution and control on carbonate deposition in northeast Australia: Global and Planetary Change, v. 3, no. 4, p. 341-361.

FRICKE H., E. B., JOHNSON K. R. AND JAHREN A. H. (2007) 2007, Carbon isotope characterization of the closed canopy (?) Castle Rock rainforest, *in* America, G. S. o., ed., Abstracts with Programs, Volume 39: Colorado Convention Center, p. 27.

GRAHAM, H. V., PATZKOWSKY, M. E., WING, S. L., PARKER, G. G., FOGEL, M. L., AND FREEMAN, K. H., 2014, Isotopic characteristics of canopies in simulated leaf assemblages: Geochimica et Cosmochimica Acta, v. 144, p. 82-95.

GREENWOOD, D., 1994, Palaeobotanical evidence for Tertiary climates: History of the Australian vegetation cretaceous to recent, p. 44-59.

GREENWOOD, D. R., MOSS, P. T., ROWETT, A. I., VADALA, A. J., AND KEEFE, R. L., 2003, Plant communities and climate change in southeastern Australia during the early Paleogene: SPECIAL PAPERS-GEOLOGICAL SOCIETY OF AMERICA, p. 365-380.

GREIN, M., ROTH-NEBELSICK, A., AND WILDE, V., 2010, Carbon isotope composition of middle Eocene leaves from the Messel Pit, Germany: Palaeodiversity, v. 3, p. 1-7.

HILL, R., AND CARPENTER, R., 1991, Evolution of Acmopyle and Dacrycarpus (Podocarpaceae) foliage as inferred from macrofossils in south-eastern Australia: Australian systematic botany, v. 4, no. 3, p. 449-479.

HILL, R. S., 1988, Tertiary Isoetes from Tasmania: Alcheringa, v. 12, no. 2, p. 157-162.

HILL, R. S.,, 1989, New species of Phyllocladus (Podocarpaceae) macrofossils from southeastern Australia: Alcheringa, v. 13, no. 3, p. 193-208.

HILL, R. S.,, 1991, Leaves of Eucryphia (Eucryphiaceae) from Tertiary sediments in south-eastern Australia: Australian Systematic Botany, v. 4, no. 3, p. 481-497.

HILL, R. S.,, 1994, History of the Australian vegetation: Cretaceous to Recent, Cambridge University Press.

HILL, R. S., AND CHRISTOPHEL, D. C., 2001, Two new species of Dacrydium (Podocarpaceae) based on vegetative fossils from Middle Eocene sediments at Nelly Creek, South Australia: Australian Systematic Botany, v. 14, no. 2, p. 193-205.

HILL, R. S., AND MACPHAIL, M. K., 1983, Reconstruction of the Oligocene vegetation at Pioneer, northeast Tasmania: Alcheringa, v. 7, no. 4, p. 281-299.

HILL, R. S., AND PAULL, R., 2003, Fitzroya (Cupressaceae) macrofossils from Cenozoic sediments in Tasmania, Australia: Review of Palaeobotany and Palynology, v. 126, no. 1, p. 145-152.

JACKSON, P. C., MEINZER, F. C., GOLDSTEIN, G., HOLBROOK, N. M., CAVELIER, J., AND RADA, F., 2012, Environmental and physiological influences on carbon isotope composition of gap and understory plants in a lowland tropical forest: Stable Isotopes and Plant Carbon-Water Relations, p. 131.

JORDAN, G. J., AND MACPHAIL, M. K., 2003, A middle-late Eocene inflorescence of Caryophyllaceae from Tasmania, Australia: American Journal of Botany, v. 90, no. 5, p. 761-768.

KÖRNER, C., FARQUHAR, G., AND WONG, S., 1991, Carbon isotope discrimination by plants follows latitudinal and altitudinal trends: Oecologia, v. 88, no. 1, p. 30-40.

MACPHAIL, M., 2007, Australian Palaeoclimates: Cretaceous to Tertiary: a Review of Palaeobotanical and Related Evidence to the Year 2000. , *in* Exploration, C. R. C. f. L. E. a. M., ed., Volume Special Volume: Queensland, CRC LEME.

MACPHAIL, 2012, Palynostratigraphic analyses of outcrop samples from the Golden Fleece Rivulet macrofossil deposit, St. Helens District, North-East Tasmania: unpublished.

MACPHAIL, M., ALLEY, N., TRUSWELL, E., AND SLUITER, I., 1994, 10 Early Tertiary vegetation: evidence from spores and pollen: History of the Australian vegetation: Cretaceous to Recent, p. 189.

MACPHAIL, M. K., HILL, R. S., FORSYTH, S. M., AND WELLS, P. M., 1991, A late Oligocene-early Miocene cool climate flora in Tasmania: Alcheringa, v. 15, no. 2, p. 87-106.

MALHADO, A., MALHI, Y., WHITTAKER, R. J., LADLE, R. J., TER STEEGE, H., FABRÉ, N. N., PHILLIPS, O., LAURANCE, W. F., ARAGAO, L. E., AND PITMAN, N. C., 2012, Drip-tips are Associated with Intensity of Precipitation in the Amazon Rain Forest: Biotropica, v. 44, no. 6, p. 728-737.

MARTIN, H., 2006, Cenozoic climatic change and the development of the arid vegetation in Australia: Journal of Arid Environments, v. 66, no. 3, p. 533-563.

MARTIN, H. A., 1990, Tertiary climate and phytogeography in southeastern Australia: Review of Palaeobotany and Palynology, v. 65, no. 1, p. 47-55.

PAULL, R., AND HILL, R. S., 2003, Nothofagus kiandrensis (Nothofagaceae subgenus Brassospora), a new macrofossil leaf species from Miocene sediments at Kiandra, New South Wales: Australian Systematic Botany, v. 16, no. 4, p. 549-559.

POLE, M., 1992, Eocene vegetation from Hasties, north-eastern Tasmania: Australian systematic botany, v. 5, no. 4, p. 431-475.

POLE, M. S., AND MACPHAIL, M. K., 1996, Eocene Nypa from Regatta Point, Tasmania: Review of Palaeobotany and Palynology, v. 92, no. 1, p. 55-67.

QUILTY, P., 1994, The background: 144 million years of Australian palaeoclimate and palaeogeography: History of the Australian vegetation: Cretaceous to Recent, v. 14, p. 43.

SCHER, H. D., AND MARTIN, E. E., 2006, Timing and climatic consequences of the opening of Drake Passage: Science, v. 312, no. 5772, p. 428-430.

SCHUBERT, B. A., AND JAHREN, A. H., 2012, The effect of atmospheric CO 2 concentration on carbon isotope fractionation in C 3 land plants: Geochimica et Cosmochimica Acta, v. 96, p. 29-43.

TARRAN, M., 2013, Cenozoic Myrtaceae: Two new proposed fossil species of Kania, and palaeoclimatic implications of cuticular ecomorphologyHonours]: University of Adelaide.

TIESZEN, L. L., AND BOUTTON, T. W., 1989, Stable carbon isotopes in terrestrial ecosystem research, Stable isotopes in ecological research, Springer, p. 167-195.

TIPPLE, B. J., MEYERS, S. R., AND PAGANI, M., 2010, Carbon isotope ratio of Cenozoic CO2: a comparative evaluation of available geochemical proxies: Paleoceanography, v. 25, no. 3.

VAN DER MERWE, N. J., AND MEDINA, E., 1991, The canopy effect, carbon isotope ratios and foodwebs in Amazonia: Journal of Archaeological Science, v. 18, no. 3, p. 249-259.

WARREN, C. R., MCGRATH, J. F., AND ADAMS, M. A., 2001, Water availability and carbon isotope discrimination in conifers: Oecologia, v. 127, no. 4, p. 476-486.

WEI, W., AND THIERSTEIN, H., Upper Cretaceous and Cenozoic calcareous nannofossils of the Kerguelen Plateau (southern Indian Ocean) and Prydz Bay (East Antarctica), *in* Proceedings Barron, J., Larsen, B., et al., Proc. ODP, Sci. Results1991, Volume 119, p. 467-494.

WELLS, P., AND HILL, R., 1989, Fossil imbricate-leaved Podocarpaceae from Tertiary sediments in Tasmania: Australian systematic botany, v. 2, no. 4, p. 387-423.

ZACHOS, J., PAGANI, M., SLOAN, L., THOMAS, E., AND BILLUPS, K., 2001, Trends, rhythms, and aberrations in global climate 65 Ma to present: Science, v. 292, no. 5517, p. 686-693.

APPENDIX A: SUPPLEMENTARY DATA

ANGLESEA

Sample number	Site	Sample type	δ 13C $_{leaf}$	Corrected δ 13C $_{leaf}$	δ 13C $_{atmo}$	Δ_{leaf}	Error
AnA050	Anlgesea	Angiosperm	-33.6758	-36.22526112	-5.75	31.53731	0.52
AnA025	Anlgesea	Angiosperm	-33.4208	-35.97025993	-5.75	31.26517	0.52
AnA007	Anlgesea	Angiosperm	-33.0871	-35.63655459	-5.75	30.90925	0.52
AnA047	Anlgesea	Angiosperm	-33.053	-35.60249086	-5.75	30.87294	0.52
AnA015	Anlgesea	Angiosperm	-32.7491	-35.29857078	-5.75	30.54902	0.52
AnA037	Anlgesea	Angiosperm	-32.3354	-34.88487946	-5.75	30.10845	0.52
AnA031	Anlgesea	Angiosperm	-31.9984	-34.54787969	-5.75	29.74983	0.52
AnA002	Anlgesea	Angiosperm	-31.977	-34.52646323	-5.75	29.72705	0.52
AnA012	Anlgesea	Angiosperm	-31.9273	-34.47676349	-5.75	29.67418	0.52
AnA005	Anlgesea	Angiosperm	-31.9162	-34.46559999	-5.75	29.66231	0.52
AnA003	Anlgesea	Angiosperm	-31.8456	-34.39501584	-5.75	29.58724	0.52
AnA026	Anlgesea	Angiosperm	-31.841	-34.39040503	-5.75	29.58234	0.52
AnA027	Anlgesea	Angiosperm	-31.3959	-33.94533338	-5.75	29.10924	0.52
AnA018	Anlgesea	Angiosperm	-31.326	-33.87546202	-5.75	29.03501	0.52
AnA029	Anlgesea	Angiosperm	-31.3093	-33.8587733	-5.75	29.01729	0.52
AnA017	Anlgesea	Angiosperm	-31.239	-33.78842745	-5.75	28.94256	0.52
AnA039	Anlgesea	Angiosperm	-31.1707	-33.72017798	-5.75	28.87008	0.52
AnA046	Anlgesea	Angiosperm	-30.7842	-33.33362425	-5.75	28.45973	0.52
AnA004	Anlgesea	Angiosperm	-30.7707	-33.32013765	-5.75	28.44542	0.52
AnA009	Anlgesea	Angiosperm	-30.7632	-33.31265949	-5.75	28.43749	0.52
AnA019	Anlgesea	Angiosperm	-30.6451	-33.19449654	-5.75	28.31212	0.52
AnA049	Anlgesea	Angiosperm	-30.5797	-33.12911205	-5.75	28.24277	0.52
AnA008	Anlgesea	Angiosperm	-30.4603	-33.00971775	-5.75	28.11614	0.52
AnA010	Anlgesea	Angiosperm	-30.0697	-32.61910333	-5.75	27.7021	0.52
AnA034	Anlgesea	Angiosperm	-29.9773	-32.5267348	-5.75	27.60424	0.52

AnA023	Anlgesea	Angiosperm	-29.8322	-32.38164606	-5.75	27.45056	0.52
AnA038	Anlgesea	Angiosperm	-29.737	-32.28645218	-5.75	27.34975	0.52
AnA055	Anlgesea	Angiosperm	-29.5036	-32.05303632	-5.75	27.10266	0.52
AnA043	Anlgesea	Angiosperm	-29.3937	-31.94318892	-5.75	26.98642	0.52
AnA001	Anlgesea	Angiosperm	-29.3454	-31.89483056	-5.75	26.93526	0.52
AnA006	Anlgesea	Angiosperm	-29.2361	-31.7855337	-5.75	26.81964	0.52
AnA040	Anlgesea	Angiosperm	-29.1774	-31.72684356	-5.75	26.75756	0.52
AnA020	Anlgesea	Angiosperm	-29.0335	-31.58296572	-5.75	26.60541	0.52
AnA045	Anlgesea	Angiosperm	-28.8936	-31.44305784	-5.75	26.45751	0.52
AnA011	Anlgesea	Angiosperm	-28.631	-31.18043632	-5.75	26.18	0.52
AnA016	Anlgesea	Angiosperm	-28.6167	-31.16611528	-5.75	26.16487	0.52
AnA032	Anlgesea	Angiosperm	-28.1348	-30.68426555	-5.75	25.6561	0.52
AnA035	Anlgesea	Angiosperm	-28.0066	-30.5560188	-5.75	25.52077	0.52
AnA014	Anlgesea	Angiosperm	-27.9017	-30.45112041	-5.75	25.4101	0.52
AnA053	Anlgesea	Angiosperm	-27.8283	-30.37773666	-5.75	25.3327	0.52
AnA051	Anlgesea	Angiosperm	-27.6919	-30.2413371	-5.75	25.18886	0.52
AnA028	Anlgesea	Angiosperm	-27.5715	-30.12097458	-5.75	25.06197	0.52
AnG043	Anlgesea	Gymnosperm	-25.4249	-27.97433375	-5.75	22.80413	0.52
AnG044	Anlgesea	Gymnosperm	-25.6302	-28.1796296	-5.75	23.01963	0.52
AnG034	Anlgesea	Gymnosperm	-26.2956	-28.84504277	-5.75	23.71874	0.52
AnG039	Anlgesea	Gymnosperm	-27.1657	-29.71517249	-5.75	24.63438	0.52
AnG007	Anlgesea	Gymnosperm	-27.6819	-30.23134074	-5.75	25.17832	0.52
AnG017	Anlgesea	Gymnosperm	-27.758	-30.30744316	-5.75	25.25857	0.52
AnG008	Anlgesea	Gymnosperm	-27.9875	-30.53692171	-5.75	25.50062	0.52
AnG002	Anlgesea	Gymnosperm	-28.2486	-30.79802251	-5.75	25.77616	0.52
AnG010	Anlgesea	Gymnosperm	-28.6351	-31.18452611	-5.75	26.18432	0.52
AnG024	Anlgesea	Gymnosperm	-28.835	-31.38442847	-5.75	26.39554	0.52
AnG009	Anlgesea	Gymnosperm	-28.8988	-31.44824587	-5.75	26.46299	0.52
AnG006	Anlgesea	Gymnosperm	-29.2702	-31.81965469	-5.75	26.85573	0.52
AnG021	Anlgesea	Gymnosperm	-29.2905	-31.8399923	-5.75	26.87724	0.52
AnG040	Anlgesea	Gymnosperm	-29.4303	-31.97971617	-5.75	27.02507	0.52

AnG032	Anlgesea	Gymnosperm	-30.0086	-32.55800553	-5.75	27.63736	0.52
AnG016	Anlgesea	Gymnosperm	-31.6595	-34.2089712	-5.75	29.38943	0.52
AnDC003	Anlgesea	D.Cuticle	-29.1708	-31.72019689	-5.75	26.75053	0.52
AnDC002	Anlgesea	D.Cuticle	-28.8858	-31.43526449	-5.75	26.44927	0.52
AnDC001	Anlgesea	D.Cuticle	-28.6726	-31.22207704	-5.75	26.22399	0.52
AnDC005	Anlgesea	D.Cuticle	-28.2317	-30.7810945	-5.75	25.75829	0.52
AnDC004	Anlgesea	D.Cuticle	-28.2226	-30.77201868	-5.75	25.74871	0.52

KIANDRA

Sample number	**Site**	**Sample type**	**δ 13C $_{leaf}$**	**Corrected δ 13C $_{leaf}$**	**δ 13C $_{atmo}$**	**Δ_{leaf}**	**Error**
KA017	Kiandra	Angiosperm	-32.3778	-35.0273	-5.65	30.36026	0.516
KA015	Kiandra	Angiosperm	-32.2458	-34.8952	-5.65	30.21967	0.516
KA029	Kiandra	Angiosperm	-31.9942	-34.6437	-5.65	29.95195	0.516
KA090	Kiandra	Angiosperm	-31.8308	-34.4802	-5.65	29.77811	0.516
KA051	Kiandra	Angiosperm	-31.1271	-33.7765	-5.65	29.03016	0.516
KA025	Kiandra	Angiosperm	-31.0371	-33.6865	-5.65	28.93454	0.516
KA013	Kiandra	Angiosperm	-30.8891	-33.5386	-5.65	28.7775	0.516
KA028	Kiandra	Angiosperm	-30.861	-33.5104	-5.65	28.74762	0.516
KA034	Kiandra	Angiosperm	-30.7465	-33.396	-5.65	28.62614	0.516
KA026	Kiandra	Angiosperm	-30.6485	-33.298	-5.65	28.52211	0.516
KA032	Kiandra	Angiosperm	-30.6171	-33.2665	-5.65	28.48874	0.516
KA031	Kiandra	Angiosperm	-30.5258	-33.1753	-5.65	28.39194	0.516
KA011	Kiandra	Angiosperm	-30.5184	-33.1679	-5.65	28.3841	0.516
KA047	Kiandra	Angiosperm	-30.4838	-33.1332	-5.65	28.34733	0.516
KA002	Kiandra	Angiosperm	-30.406	-33.0554	-5.65	28.26487	0.516
KA035	Kiandra	Angiosperm	-30.3545	-33.0039	-5.65	28.21022	0.516
KA033	Kiandra	Angiosperm	-30.2195	-32.869	-5.65	28.06713	0.516
KA008	Kiandra	Angiosperm	-30.075	-32.7244	-5.65	27.91392	0.516
KA040	Kiandra	Angiosperm	-29.9367	-32.5861	-5.65	27.76739	0.516

KA043	Kiandra	Angiosperm	-29.9226	-32.5721	-5.65	27.75248	0.516
KA046	Kiandra	Angiosperm	-29.922	-32.5715	-5.65	27.75186	0.516
KA050	Kiandra	Angiosperm	-29.8829	-32.5323	-5.65	27.71041	0.516
KA006	Kiandra	Angiosperm	-29.8235	-32.4729	-5.65	27.64749	0.516
KA009	Kiandra	Angiosperm	-29.7866	-32.4361	-5.65	27.60842	0.516
KA001	Kiandra	Angiosperm	-29.7713	-32.4208	-5.65	27.59221	0.516
KA045	Kiandra	Angiosperm	-29.7555	-32.4049	-5.65	27.57546	0.516
KA027	Kiandra	Angiosperm	-29.7168	-32.3662	-5.65	27.53443	0.516
KA044	Kiandra	Angiosperm	-29.5407	-32.1902	-5.65	27.34806	0.516
KA055	Kiandra	Angiosperm	-29.4889	-32.1384	-5.65	27.29323	0.516
KA065	Kiandra	Angiosperm	-29.4566	-32.1061	-5.65	27.25904	0.516
KA052	Kiandra	Angiosperm	-29.2861	-31.9355	-5.65	27.07853	0.516
KA037	Kiandra	Angiosperm	-29.2431	-31.8926	-5.65	27.03309	0.516
KA004	Kiandra	Angiosperm	-29.1971	-31.8465	-5.65	26.98438	0.516
KA049	Kiandra	Angiosperm	-29.1403	-31.7897	-5.65	26.92429	0.516
KA036	Kiandra	Angiosperm	-29.0571	-31.7066	-5.65	26.83637	0.516
KA041	Kiandra	Angiosperm	-29.0004	-31.6499	-5.65	26.77641	0.516
KA020	Kiandra	Angiosperm	-28.948	-31.5974	-5.65	26.72092	0.516
KA018	Kiandra	Angiosperm	-28.9036	-31.553	-5.65	26.67401	0.516
KA024	Kiandra	Angiosperm	-28.8797	-31.5292	-5.65	26.64877	0.516
KA021	Kiandra	Angiosperm	-28.845	-31.4944	-5.65	26.61206	0.516
KA042	Kiandra	Angiosperm	-28.8244	-31.4738	-5.65	26.59028	0.516
KA053	Kiandra	Angiosperm	-28.6949	-31.3443	-5.65	26.45337	0.516
KA007	Kiandra	Angiosperm	-28.6552	-31.3046	-5.65	26.41145	0.516
KA012	Kiandra	Angiosperm	-28.5597	-31.2092	-5.65	26.3106	0.516
KA019	Kiandra	Angiosperm	-28.3509	-31.0004	-5.65	26.09004	0.516
KA098	Kiandra	Angiosperm	-28.3195	-30.9689	-5.65	26.05684	0.516
KA016	Kiandra	Angiosperm	-28.2302	-30.8796	-5.65	25.96253	0.516
KA030	Kiandra	Angiosperm	-28.1893	-30.8387	-5.65	25.91939	0.516
KA054	Kiandra	Angiosperm	-28.0489	-30.6984	-5.65	25.77123	0.516
KA023	Kiandra	Angiosperm	-27.7844	-30.4338	-5.65	25.49208	0.516

KA038	Kiandra	Angiosperm	-26.9375	-29.587	-5.65	24.59964	0.516
KA003	Kiandra	Angiosperm	-26.2256	-28.875	-5.65	23.85053	0.516
KDC002	Kiandra	D.Cuticle	-28.2655	-30.9149	-5.65	25.9998	0.516
KDC001	Kiandra	D.Cuticle	-29.0094	-31.6589	-5.65	26.78591	0.516
KDC003	Kiandra	D.Cuticle	-28.3961	-31.0455	-5.65	26.13775	0.516
KDC004	Kiandra	D.Cuticle	-29.029	-31.6784	-5.65	26.80656	0.516
KDC005	Kiandra	D.Cuticle	-28.7279	-31.3774	-5.65	26.48834	0.516

NELLY CREEK *MYRT* TEST

Sample number	**Site**	**Sample type**	**δ 13C $_{leaf}$**
10a	Nelly Creek	treated	-27.9474964
9a	Nelly Creek	treated	-26.85757337
8a	Nelly Creek	treated	-28.2617897
7a	Nelly Creek	treated	-27.48433357
6a	Nelly Creek	treated	-28.88718863
5a	Nelly Creek	treated	-27.5893972
4a	Nelly Creek	treated	-27.25567688
3a	Nelly Creek	treated	-27.2094029
2a	Nelly Creek	treated	-27.59049921
1a	Nelly Creek	treated	-27.78128442
10b	Nelly Creek	non treated	-
9b	Nelly Creek	non treated	-28.41027905
8b	Nelly Creek	non treated	-29.4592062
7b	Nelly Creek	non treated	-29.87669373
6b	Nelly Creek	non treated	-29.65253393
5b	Nelly Creek	non treated	-28.92460364
4b	Nelly Creek	non treated	-28.66563327
3a	Nelly Creek	non treated	-28.85872504
2b	Nelly Creek	non treated	-29.48530052

1b	Nelly Creek	non treated	-28.60265503

DISPERSED CUTICLE

Sample number	Site	Sample type	δ 13C $_{leaf}$	Corrected δ 13C $_{leaf}$	δ 13C $_{atmo}$	Δ	Error
RPDC001	Regatta Point	D.Cuticle	-27.16	-29.54	-5.925	24.33205	0.125
RPDC002	Regatta Point	D.Cuticle	-27.00	-29.37	-5.925	24.15822	0.125
NCDC001	Nelly Creek	D.Cuticle	-27.58	-29.93	-5.95	24.71794	0.35
NCDC002	Nelly Creek	D.Cuticle	-28.36	-30.71	-5.95	25.5489	0.35
GGDC001	Golden Grove	D.Cuticle	-27.53	-30.08	-5.75	25.08614	0.25
GGDC002	Golden Grove	D.Cuticle	-27.71	-30.26	-5.75	25.27392	0.25
LADC001	Loch Aber	D.Cuticle	-25.09	-27.64	-5.75	22.50979	0.25
LADC002	Loch Aber	D.Cuticle	-25.11	-27.66	-5.75	22.53238	0.25
HDC001	Hasties	D.Cuticle	-27.31	-29.71	-5.9	24.53491	0.4
HDC002	Hasties	D.Cuticle	-27.21	-29.61	-5.9	24.43554	0.4
LRRDC002	Little Rapid River	D.Cuticle	-26.40	-28.55	-6.15	23.05353	0.15
LRDC001	Little Rapid River	D.Cuticle	-25.65	-27.80	-6.15	22.27325	0.15
LRDC002	Lea River	D.Cuticle	-25.94	-28.09	-6.15	22.57665	0.15
LRRDC001	Lea River	D.Cuticle	-27.17	-29.32	-6.15	23.87098	0.15
GFDC001	Golden Fleece	D.Cuticle	-28.67	-30.82	-6.15	25.45693	0.25
GFDC002	Golden Fleece	D.Cuticle	-29.83	-31.98	-6.15	26.67898	0.25
PDC001	Pioneer	D.Cuticle	-27.84	-30.24	-5.9	25.09516	0.5
PDC002	Pioneer	D.Cuticle	-27.85	-30.25	-5.9	25.11005	0.5
MDC001	Monpealyata	D.Cuticle	-25.51	-27.81	-6	22.43663	0.2
MDC002	Monpealyata	D.Cuticle	-24.95	-27.25	-6	21.84877	0.2

APPENDIX C: COMPLETE PROCEDURE

PILOT STUDY

Nelly Creek samples, prepared by Myall Tarran
Large leaves found in Nelly Creek samples (faint aroma of ethanol)
Leaves cut in half
Both fragments placed in deionised water
Placed individually into Eppendorf tubes
One half treaded with CrO_3 (chromic (III) acid) (20%) for ~18hrs (until they were clear / pale yellow) in the fume hood
Then rinsed with deionised water 5 times
And brushed with fine tipped paint brushes to remove of excess dirt / tissue / acid
Samples then placed in to 40°C drying oven to evaporate excess water for ~48hrs

Samples then homogenised following procedure:
Wipe down work surface with water to clean off the surface
Work implements are rinsed with solvents in order of, methanol, dichloromethane, and hexane, in a fume hood
Allow solvent-rinsed implements to air dry in the hood
Plant tissue is ground in Eppendorf tubes, with glass-stirring rod
Between grinding samples the implements are rinsed (as described above)

Samples then weighed following:
(Balance, Spatulas, Tweezers, Ethanol, Cleaning Plate, Tin Boats,
Wipe down work surface with ethanol to clean off the surface
Work implements are rinsed with ethanol
Tweezers place tin boats on balance
Once settled, the balance is reset while tin boat is in place
Small amounts of the homogenized sample is placed in the tin boat, using the spatula, until the sample container is empty, or the balance has reached 0.5-1.5mg
Record value
The tin boat is then folded, firstly folded from the top to hold the sample in, then from the sides until a compact cube is reached
Place crimped sample into isotope plate
Cleaning plate and instruments are then rinsed with ethanol and repeated for each sample

Weighting of the standards also follow the above procedure, for the following standards glutamic acid, and glycine

4 Duplicates were made

Criteria for angiosperm / gymnosperm determination under dissecting microscope
Angiosperms:

Stomates arranged in areoles,
Complex vein networks,
Random cell arrangement,
Gymnosperms
Linear arrangements of cells,
Linear veins
Linear arrangement of stomates

Plan: 50 angiosperms
50 gymnosperms
2 dispersed cuticle (duplicated 8 times)
Placed into individual vials
Individually photographed
Individual cuticle slides

Labelled example AnA001
An ~ Anlgesea
A ~Angiosperm
G ~Gymnosperm
DC ~Dispersed Cuticle
#001 ~Sample number

ANGLESEA EXPERIMENT

Individual leaf
Jar A of dispersed cuticle emptied into tube
Excess ethanol siphoned off into separate beakers
Excess ethanol purified via filtration
Then replaced into original DC jar
Picking procedure after ethanol clears
Large fragments (>1mm^2) placed into small beaker filled with deionised water
>50 fragments were picked
Pipetted off water in beaker
Filled beaker with CrO_3 (10%) until just covering samples, in fume hood
Left over night, for ~18hrs (relatively clean), in fume hood
Samples cleaned by pipetting of CrO_3 (10%) into waste container, in fume hood
Rinsed samples with deionised water 5 times, pipetting off water each time, in fume hood
Place into wash glass and cleaned (under dissecting microscope) with fine tipped paintbrushes, and pins
Picked based on above criteria, and placed into individual vials

Remaining dispersed cuticle then replaced back into Jar A

Photography:

Samples individually placed into wash glass, full of deionised water
Picture taken using Niko D3000 with a 60mm AF-5 Micro Nikkor lens
Sample placed back into vial, no water
Vial is filled with CrO_3 (10%), until covering sample, in fume hood
Left for 48hrs in fume hood
CrO_3 (10%), pipetted out in fume hood
Rinsed with deionised water 5 times in fume hood
Samples then placed into 40°C drying oven to evaporate excess water for ~48hrs

Dispersed cuticle
Dispersed cuticle Jar C, mixed with metal spoon
Modified needle nose pipette, with end cut off, used to siphon off cuticle fragments
Pipetted into vial
Ethanol is pipetted out, with separate pipette
More siphoned cuticle is placed into same vial
Repeated until ½ cm in the bottom of vial
All ethanol is pipetted out
Rinsed 5 times with deionised water
CrO_3 (10%) is pipetted in, until covering the samples, in fume hood
Left for 50 hrs in fume hood
Rinsed 5 times with deionised water, in fume hood
Samples then placed into 40°C drying oven to evaporate excess water for ~48hrs

Note: three samples types were taken: 50 gymnosperms and 50 angiosperms, and 5 dispersed cuticle

Homogenised and weighted following the pilot procedures

KIANDRA EXPERIMENT

Individual leaf
Some large dried leaves, from previous work, placed into small beaker with deionised water
Dispersed cuticle Jar C,
Larger spoon full of dispersed cuticle is placed into wash glass
Picking procedure after ethanol clears
Large fragments (>1mm^2) placed into small beaker filled with deionised water
>50 fragments were picked
Pipetted off water in beaker
Filled beaker with CrO_3 (10%) until just covering sample, in fume hood
Left over night, for ~18hrs (relatively clean), in fume hood
Samples cleaned by pipetting of CrO_3 (10%) into waste container, in fume hood
Rinsed samples with deionised water 5 times, pipetting off water each time, in fume hood
Place into wash glass and cleaned (under dissecting microscope) with fine tipped paintbrushes, and pins
Picked based on above criteria, and placed into individual vials

Remaining dispersed cuticle then replaced back into Jar C

Dispersed cuticle
Dispersed cuticle Jar C, mixed with metal spoon
Modified needle nose pipette, with end cut off, used to siphon off cuticle fragments
Pipetted into vial
Ethanol is pipetted out, with separate pipette
More siphoned cuticle is placed into same vial
Repeated until ½ cm in the bottom of vial
All ethanol is pipetted out
Rinsed 5 times with deionised water
CrO_3 (10%) is pipetted in, until covering the samples, in fume hood
Left for 50 hrs in fume hood
Rinsed 5 times with deionised water, in fume hood
Samples then placed into 40°C drying oven to evaporate excess water for ~48hrs

Photography:
Samples individually placed into wash glass, full of deionised water
Picture taken using Niko D3000 with a 60mm AF-5 Micro Nikkor lens
Sample placed back into vial, no water
Vial is filled with CrO_3 (10%), until covering sample
Left for 48hrs
CrO_3 (10%), pipetted out
Rinsed with deionised water 5 times
Samples then placed into 40°C drying oven to evaporate excess water for ~48hrs

Note: only two sample types were taken: 60 angiosperms and 5 dispersed cuticle

Homogenised and weighted following the pilot procedures

DISPERSED CUTICLE EXPERIMENT

The 12 fossil sites had dispersed cuticle jars, some had been stored in ethanol and others were completely dry. The following two procedures are for the wet and dry jars.

Wet cuticles
Dispersed cuticle Jar for site x, mixed with metal spoon
Modified needle nose pipette, with end cut off, used to siphon off cuticle fragments
Pipetted into vial
Ethanol is pipetted out, with separate pipette
More siphoned cuticle is placed into same vial
Repeated until ½ cm in the bottom of vial
All ethanol is pipetted out
Rinsed 5 times with deionised water

CrO_3 (10%) is pipetted in, until covering the samples, in fume hood
Left for 50 hrs in fume hood
Rinsed 5 times with deionised water, in fume hood
Samples then placed into 40°C drying oven to evaporate excess water for ~48hrs

Dry cuticles

Dispersed cuticle Jar for site x,
Needle nosed tweezers used to pick cuticle fragments
Placed in the vial until ½ cm in the bottom of vial
Rinsed 5 times with deionised water
CrO_3 (10%) is pipetted in, until covering the samples, in fume hood
Left for 50 hrs in fume hood
Rinsed 5 times with deionised water, in fume hood
Samples then placed into 40°C drying oven to evaporate excess water for ~48hrs