Abrupt plant physiological changes in southern New Zealand at the termination of the Mi-1 event reflect shifts in hydroclimate and $p\text{CO}_2$

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Abstract

A rise in atmospheric CO_2 is believed to be necessary for the termination of large-scale glaciations. Although the Antarctic Ice Sheet is estimated to have melted from ~125% to ~50% its modern size, there is thus far no evidence for an increase in atmospheric CO_2 associated with the Mi-1 glacial termination in the earliest Miocene. Here, we present evidence from a high-resolution terrestrial record of leaf physiological change in southern New Zealand for an abrupt increase in atmospheric CO_2 coincident with the termination of the Mi-1 glaciation and lasting approximately 20 kyr. Quantitative $p\text{CO}_2$ estimates, made using a leaf gas exchange model, suggest that atmospheric CO_2 levels may have doubled during this period, from 516 ± 111 ppm to 1144 ± 410 ppm, and subsequently returned back to 425 ± 53 ppm. The 20-kyr interval with high $p\text{CO}_2$ estimates is also associated with a period of increased moisture supply to southern New Zealand, inferred from carbon and hydrogen isotopes of terrestrial leaf waxes. The results provide the first high-resolution record of terrestrial environmental change at the Oligocene/Miocene boundary, document a ~20 kyr interval of elevated $p\text{CO}_2$ and increased local

moisture availability, and provide insight into ecosystem response to a major orbitally driven climatic transition.

Keywords: leaf physiology; carbon dioxide; precipitation; Oligocene/Miocene; deglaciation

1. Introduction

The Oligocene/Miocene boundary (23.03 Ma) is associated with a period of Antarctic glacial expansion, known as the Mi-1 glaciation (Pälike et al., 2006; Wilson et al., 2008; Liebrand et al., 2011; Mawbey and Lear, 2013). The Mi-1 glaciation lasted approximately 400 kyr, beginning with ice-sheet growth in the latest Oligocene, followed by stability across the Oligocene/Miocene boundary, and finally, ice sheet retreat in the earliest Miocene (Pälike et al., 2006; Liebrand et al., 2011). At its maximum extent, Antarctic ice volume during Mi-1 is estimated to have been 125% its current size, after growing from 50% its current size in the late Oligocene (Pekar and DeConto, 2006). Despite these sizeable changes and their global climatic consequences, identifying the mechanisms responsible for the inception and termination of the Mi-1 glaciation has been challenging. The transience of the Mi-1 glaciation suggests that changes in orbital configuration resulted in climatic threshold responses, likely achieved through internal climate feedbacks such as albedo changes, chemical weathering and carbon cycling, changes in ocean circulation, and changes in atmospheric CO₂ (Lear et al., 2004; Wilson et al., 2008; Pollard and DeConto, 2009; Liebrand et al., 2011; Mawbey and Lear, 2013). Modelling studies indicate that atmospheric CO₂ must have played an important role in terminating Antarctic glaciations such as Mi-1 (DeConto and Pollard, 2003; DeConto et al., 2008). However, paleo-*p*CO₂ estimates based on marine proxies suggest that atmospheric CO₂ stayed below the

level required to terminate the Mi-1 glaciation (DeConto et al., 2008; Zhang et al., 2013). Fossil plants from terrestrial archives have the potential to record short-term atmospheric CO₂ variability (e.g. Woodward, 1987); however, Oligocene/Miocene terrestrial archives often lack the temporal resolution and age control necessary to target a particular event such as the Mi-1 termination (Kürschner and Kvaček, 2009; Roth-Nebelsick et al., 2014).

The Foulden Maar diatomite is a lake deposit in southern New Zealand that accumulated in a phreatomagmatic explosion crater (Lindqvist and Lee, 2009). The eruptive event and establishment of the lake basin occurred in the latest Oligocene (Lindqvist and Lee, 2009; Fox et al., 2015). Atop the breccia that rapidly collapsed into the newly formed crater, lacustrine diatomaceous sediments accumulated for at least a ~100 kyr period. Annual laminations (i.e. varves) characterize the entirety of the diatomite (~100m of sediment) and provide floating chronological control within the depositional sequence (Lindqvist and Lee, 2009; Fox et al., 2016). A magnetic reversal to normal polarity occurred near the base of the sediment sequence and polarity remained normal for the remaining period of deposition (i.e., at least 100 kyrs; Fox et al., 2016); thus, the onset of diatomite sedimentation is constrained to normal chron C6Cn.2n, which is currently dated to 23.03 Ma BP and defines the O/M boundary (Billups et al., 2004; Wilson et al., 2008). The environment at Foulden Maar during the time of deposition was humid subtropical (Reichgelt et al., 2013) and a diverse Lauraceae-dominated rainforest surrounded the ancient lake (Bannister et al., 2012). The great depth of the lake relative to its area (max depth ~ 100 m; diameter ~1.5 km) resulted in bottom-water anoxia (Lindqvist and Lee, 2009; Reichgelt et al., 2013), and as a consequence the quality of preservation in the Foulden Maar deposits is extremely high, preserving anatomic detail and organic material of both plants and animals (Lindqvist and Lee, 2009).

The early Miocene lake at Foulden Maar was optimally located to record changes in the climate of the Southern Ocean, because southern New Zealand is one of the few landmasses extending to southern mid-latitudes. Furthermore, New Zealand's climate is sensitive to changes in the strength of the Antarctic Circumpolar Current, which is influenced by Antarctic ice-extent (Carter et al., 2009). In this study, we examine aspects of the terrestrial ecosystem at Foulden Maar during the termination of the Mi-1 glaciation. We quantify changes in the leaf micromorphology of a single species of the Lauraceae family (*Litsea calicarioides*) preserved in the Foulden Maar sedimentary sequence, and interpret these changes as a proxy for past atmospheric CO₂. We also compare the micromorphological parameters of the fossil leaves to those of a century-long collection of the closest living relative of this plant (*Litsea calicaris*). We report bulk sediment carbon isotopes ($\delta^{13}\text{C}$ values), sedimentary leaf-wax $\delta^{13}\text{C}$ values and hydrogen isotopes (δD values), which we interpret as signatures of past ecosystem water use efficiency, and therefore moisture availability.

2. Materials & Methods

2.1. Geological setting

The Foulden Maar Diatomite (45.5270°S; 170.2191°E; Fig. 1c) is part of the Waipiata Volcanic Group (Lindqvist and Lee, 2009), which comprises remnants of a volcanic field that was active 23.6–8.9 Ma in central and coastal Otago, southern New Zealand (Coombs et al., 2008). The Waipiata Volcanic Field features numerous eroded scoria cones, pyroclastic infills, and ejecta rims as part of maar-diatreme complexes (Németh and White, 2003). Maar-diatreme formation requires proximity to and/or intersection with the water table. A post-formative ejecta rim surrounding the resulting crater usually prevents surface water inflow (White and Ross, 2011),

and such lakes are typically an expression of the local groundwater table. The Foulden Maar crater formed explosively due to pressure buildup as groundwater interacted with an intraplate magma chamber (Lindqvist and Lee, 2009). There is no evidence for external inflow of surface water in the Foulden Maar Diatomite (Fox et al., 2015). A ~180 m drillcore was recovered from the site, of which ~120 m consists of laminated diatomite (Fox et al., 2015). The continuity of the diatomite is episodically interrupted by turbidites (Lindqvist and Lee, 2009) rich in plant material, which probably represent lakeside slumping; rapidly depositing plant material in the center of the lake (Fox et al., 2015).

2.2. Fossil leaves

This paper concerns fossil leaves of *Litsea calicarioides* recovered from Foulden Maar (Lauraceae) (Bannister et al., 2012). Leaves of *L. calicaroides* (Fig. 2) are distinguishable from other Lauraceous leaves of Foulden Maar because the stomata have prominent polar rods, a distinct peristomatal rim, and lack the broad stomatal scales characteristic of many other Lauraceae species (Bannister et al., 2012). Leaf beds are not evenly distributed throughout the length of the core and *L. calicaroides* leaves have uneven representation throughout the core; therefore the recovery of *L. calicarioides* leaves reported here is unevenly distributed with respect to core depth. A total of 62 fossil leaves from 19 intervals were recovered (Appendix C, Fig. C1).

2.3. Modern leaves

Today the genus *Litsea* is represented in New Zealand by the endemic *L. calicaris* (mangeao), which is restricted to the northern regions of the North Island (Fig. 1b, c). Throughout most of its

range it represents a localized subcanopy component, although in certain soil types it may be the dominant canopy component (de Lange, 2011). Fossil *L. calicarioides* derives its name from its strong morphological similarity to *L. calicaris* (Fig. 2) (Bannister et al., 2012). We established a record of leaf physiological parameters of *L. calicaris* for the past century, during which time anthropogenic emissions have increased atmospheric CO₂ from 295 to 390 ppm (Appendix A, Table A3). A total of 27 leaves were obtained from the Auckland War Memorial Museum Herbarium, spanning 1900–2012 and representing a large part of the geographical distribution of *L. calicaris* (Fig. 1b).

2.4. Leaf preparation

Fossil and modern leaves were dissected by soaking leaves in 40% hydrogen peroxide (H₂O₂) and tetra-sodium pyrophosphate salt (Na₄P₂O₇ · 10[H₂O]) and heated to 60 °C. Modern leaf cuticles were soaked for several days, whereas fossil leaves were soaked for a few hours to avoid potential disintegration of the fossil leaf cuticle. After adaxial and abaxial leaf cuticle were separated from the leaf epidermis, the material was cleaned using small paintbrushes and water, and the leaf material was stained with <0.5% Crystal Violet (C₂₅N₃H₃₀Cl) and mounted on glass slides with glycerin jelly.

2.5. Sediment sampling

Sediment samples were collected from the Foulden Maar sediment cores by integrating ~2,000 couplets (i.e. varves) into a single sample. This was accomplished by transverse sampling of a freshly exposed core surface using solvent-washed metal utensils. Turbidites encountered during

sampling transects were not collected. Thusly, 50 sediment samples each representing 2,000-yrs of deposition across a ~100,000 yr interval were collected.

2.6. Isotope analysis

Sediment samples were freeze-dried, homogenized, and lipid-extracted using an ASE350 (Dionex) with a 9:1 mixture (v/v) of dichloromethane:methanol (3 cycles) at 100 °C and 1700psi. Total lipid extracts were separated into acid and neutral fractions by flash chromatography over 3-aminopropyl-functionalized silica-gel. The acid fraction was transesterified using acidified methanol and, to further purify the resulting fatty acid methyl esters, hydroxyl acids were removed by flash silica-gel chromatography (dichloromethane as eluent).

Purified fatty acid methyl esters were identified and quantified using a gas chromatograph - mass selective detector (Agilent 5975C). Hydrogen and carbon isotope ratios of fatty acid methyl esters were determined by gas chromatography-isotope ratio mass spectrometry (Thermo Delta V Plus coupled to a Trace GC through GC Isolink). $^2\text{H}/^1\text{H}$ ($^{13}\text{C}/^{12}\text{C}$) of analytes were measured relative to $^2\text{H}/^1\text{H}$ ($^{13}\text{C}/^{12}\text{C}$) of reference H_2 (CO_2) gas, the isotopic composition of which was determined on the VSMOW (VPDB) scale according to the identical treatment principle, by analysis of molecular compounds of known isotopic composition (Mix A, Arnt Schimmelmann, Indiana University). Isotope values for fatty acid methyl esters were corrected for the contribution from the methyl group added during derivatization (Polissar and D'Andrea, 2014). Analytical uncertainty of leaf wax $\delta^{13}\text{C}$ and δD values was determined using pooled standard deviations of replicates (Polissar and D'Andrea, 2014) and the uncertainty from calibration to VPDB and VSMOW scales, respectively, was determined using molecular standards (Mix A,

Arndt Schimmelmann, Indiana University) in the manner recommended by Polissar and D'Andrea (2014).

Bulk sediment $\delta^{13}\text{C}$ were measured using a Costech elemental combustion system (EA) coupled to a Delta V Plus IRMS (Thermo). To place measured values on the VPDB scale, a two-point isotope calibration curve was constructed using standards USGS40 and USGS41. A third standard (USGS24) was run during the period of data acquisition to evaluate the accuracy of measurement. Pooled standard deviations of replicate measurements were used to calculate measurement uncertainty.

2.7. Leaf physiology interpretation

Plant metabolic processes favor the light stable isotope of carbon (^{12}C) over the heavier isotope (^{13}C). Therefore, carbon fixed in plant organic material has a lower $^{13}\text{C}/^{12}\text{C}$ ratio than that of the atmospheric CO_2 used for photosynthesis (Farquhar et al., 1989). The degree of carbon isotope fractionation (Δ ; discrimination of ^{12}C over ^{13}C ; see Table 1 for a full list of abbreviations) depends on the size of the internal CO_2 reservoir (C_i) available for photosynthesis (Δ increases with C_i) and varies among plant species (Farquhar et al., 1989). The internal CO_2 reservoir used during photosynthesis occupies intercellular space after diffusion of atmospheric CO_2 (C_a) into the leaf through the stomata. The quantity of C_i is determined by the relative rates of CO_2 diffusion and photosynthetic carbon fixation (A_n) (Šantrůček et al., 2014); put another way, C_i reflects the balance between the supply and photosynthetic demand of CO_2 in a leaf. When supply is relatively high, Δ is high and plant organic material that forms has relatively low $\delta^{13}\text{C}$ value; when demand is relatively high, Δ is lower and organic material that forms has a higher

182 $\delta^{13}\text{C}$ value. If the $\delta^{13}\text{C}$ value of atmospheric CO_2 ($\delta^{13}\text{C}_{\text{air}}$) is known, then variations in $\delta^{13}\text{C}$
 183 values of plant materials ($\delta^{13}\text{C}_{\text{leaf}}$) can be used to calculate changes in Δ (Farquhar et al., 1989):

$$\Delta = \frac{\delta^{13}\text{C}_{\text{air}} - \delta^{13}\text{C}_{\text{leaf}}}{1 + \frac{\delta^{13}\text{C}_{\text{air}}}{1000}} (1)$$

184 Sources for modern and Miocene $\delta^{13}\text{C}_{\text{air}}$ can be found in Appendix A, Table A1.

185 Carbon diffusion takes place through stomata in the leaf epidermis. The areal density of
 186 stomata (SD), aperture size (a_{max}), pore depth (p_{dpt}), and the fraction of time the stomata remain
 187 open (ζ) together determine the operational rate of CO_2 conductance ($g_{\text{c[op]}}$), which is the critical
 188 supply term influencing C_i (Farquhar and Sharkey, 1982). Stomata also transpire water from the
 189 leaf to the atmosphere and plants optimize $g_{\text{c[op]}}$ to balance CO_2 diffusion into the leaf against
 190 water loss out of the leaf (Beerling and Woodward, 1995; Franks et al., 2012). SD , a_{max} , and p_{dpt}
 191 are determined for each leaf generation in response to signaling from previous leaf generations
 192 (Šantrůček et al., 2014); thus, the plant can modify these leaf physical parameters across leaf
 193 generations, but they are fixed parameters for a given leaf. However, ζ can vary in near-
 194 immediate response to environmental stimuli (Farquhar and Sharkey, 1982). The
 195 micromorphological properties SD , a_{max} , and p_{dpt} therefore determine the maximum potential
 196 stomatal conductance ($g_{\text{c[max]}}$), and together with ζ , mesophyll conductance (g_{m}), and boundary
 197 layer conductance (g_{b}), determine the operational conductance of a leaf ($g_{\text{c[op]}}$) (Farquhar and
 198 Sharkey, 1982).

199 The relationships among g_{c} , C_i , and A_n can be visualized as a series of feedback loops (Fig.
 200 3a), and are influenced by the measureable parameters SD , a_{max} , and p_{dpt} . Other measureable
 201 parameters, including Δ , epidermal cell density (ED), and leaf mass per area (LMA), also change
 202 in response to g_{c} , C_i , and A_n (Poorter et al., 2009; Šantrůček et al., 2014), but do not provide
 203 additional feedbacks (Fig. 3a). These feedback mechanisms result in the stabilization of A_n under

changing environmental conditions, such as temperature, irradiance, moisture availability, relative humidity, and atmospheric CO₂ (Fig. 3b, c). In this study, we quantify various leaf properties that reflect aspects of the carbon assimilation processes ($\delta^{13}\text{C}$, SD, a_{max} , p_{dpt} and ED) for both modern and early Miocene *Litsea* leaves (Appendix A, Table A2–9). In addition, we calculate stomatal index (SI), the fractional abundance of stomatal over epidermal cells:

$$SI = \frac{SD}{SD + ED} \times 100\% \quad (2)$$

Random areas of the leaves were photographed using 100x magnification under a light microscope, after which areas in the photographs were measured (varying between 0.06 and 0.2 mm², depending on a visual qualification of the leaf area) using the ImageJ software (Schneider et al., 2012) to calculate SD and ED. Areas on large veins were avoided for these measurements, although *Litsea calicaris* and *L. calicarioides* do display freely ending veinlets, which in some cases made the distinction between vein cells and regular epidermal cells ambiguous. Only cells that were in the area fully were counted and subsidiary cells were not included. ImageJ was also used to measure the width of the guard cells and the length of the pore and aperture. All individual measurements can be found in Appendix A, Table A9.

The leaf properties relevant to photosynthetic carbon assimilation are part of a complex system sensitive to both carbon uptake and water loss (Drake and González-Meler, 1997). Certain properties (e.g., SI and $\delta^{13}\text{C}$) have been used to determine paleoatmospheric CO₂ (C_a) concentrations (e.g. Roth-Nebelsick et al., 2014; Schubert and Jahren, 2015), with the stipulation that C_a is the primary influence controlling physiological change. Various controlled experiments, as well as herbaria-based studies, appear to support this view (e.g. Kürschner et al., 1997; Lammertsma et al., 2011; Schubert and Jahren, 2012). However, because $g_{c[\text{op}]}$ and Δ are sensitive to C_i (Šantrůček et al., 2014), the attribution of changes in leaf properties to just C_a is

controversial (Poole et al., 1996; Seibt et al., 2008; Kohn, 2016). For example, decreased moisture availability and increased C_a could lead to the same physiological leaf response (Beerling and Woodward, 1995; Drake and González-Meler, 1997): each of these environmental changes is expected to result in decreased ζ , which would diminish C_i (and result in decreased Δ), and lead to diminution of $g_{c[\max]}$ in successive leaf generation (Šantrůček et al., 2014).

It is evident that leaf physiology and phenotype are the result of numerous environmental parameters, including C_a . One method for reconstructing past C_a attempts to reconcile the inherent susceptibility of the photosynthetic process to environmental variation by modelling the possible range of g_c , A_n and C_a as an interwoven probability density function (Franks et al., 2014).

$$C_a = \frac{A_n}{g_{c[op]} \times \left(1 - \frac{C_i}{C_a}\right)} \quad (3)$$

The model relies on the assumption that the relationship among g_c , A_n , and the environment is genetically determined, and the relationship between A_n and C_a can be assessed using a modern living analogue (Franks et al., 2014; but see McElwain et al., 2016). Here we use the relationship between A_n and C_a of *Litsea calicaris* observed during the last century as an analogue for fossil *L. calicaroides* (Appendix A, Table A3 & A4).

In addition to leaf morphological measurements (SD , $a_{\max}$ and p_{dpt}) the Franks et al. (2014) model requires leaf-specific $\delta^{13}C$ measurements. Our input values for $\delta^{13}C$ do not come directly from leaf samples used for physical property analysis, because the leaves had already been slide-mounted prior to the current study, with no material reserved for isotopic analysis. In place of these leaf-specific measurements, we have used $\delta^{13}C_{wax}$ values and $\delta^{13}C_{bulk}$ values from sedimentary intervals associated with leaf bed deposition. We note that the $\delta^{13}C$ values represent an integrated signal of ecosystem $\delta^{13}C$, which could dampen the potential leaf-to-leaf variability

of individual leaf samples. However, the ecosystem-scale signal of terrestrial $\delta^{13}\text{C}_{\text{wax}}$ and $\delta^{13}\text{C}_{\text{bulk}}$ should be a good representation of the average of the sample population. Furthermore, weighted average $\delta^{13}\text{C}$ of C28, C30 and C32-fatty acids ($\delta^{13}\text{C}_{\text{wt-avg}}$) and $\delta^{13}\text{C}_{\text{bulk}}$ are nearly identical (Fig. 4a; Appendix A, Table A7), which gives us reason to believe that the absolute $\delta^{13}\text{C}$ values are a good approximation of terrestrial leaf matter. Additionally, aquatic-sourced $\delta^{13}\text{C}_{\text{wax-C16}}$ (Harwood, 1998) values are much higher (~10 ‰) (Appendix A, Table A7), suggesting that any aquatic contributions to the bulk sedimentary carbon pool should act to enrich $\delta^{13}\text{C}_{\text{bulk}}$ relative to terrestrial organic carbon. The observation that $\delta^{13}\text{C}_{\text{wt-avg}}$ and $\delta^{13}\text{C}_{\text{bulk}}$ are approximately equal implies that they each provide a good representation of the terrestrial organic matter $\delta^{13}\text{C}$. Thus, for the purposes of providing quantitative C_a estimates we used $\delta^{13}\text{C}_{\text{wt-avg}}$ and $\delta^{13}\text{C}_{\text{bulk}}$ values from the sediments and propagated the differences in these two measurements, and their analytical uncertainties, to estimate uncertainty in the $\delta^{13}\text{C}$ of terrestrial ecosystem. The input values used in the Franks et al. (2014) model to simulate $p\text{CO}_2$ can be found in Appendix A, Table A5.

L. calicaris and *L. calicarioides* bear close morphological resemblance and may be close taxonomic relatives. However, implicit in using a modern living relative to determine physiological functioning of an extinct species, is the assumption that they would respond in kind to environmental variation. Of course, this is intrinsically difficult to verify because the environmental variation causing a physiological response in a modern analogue cannot be independently measured for an extinct plant. As a means of comparing physiology in *L. calicaris* and *L. calicarioides* we performed principal component analysis (PCA) on the measured micromorphological properties of fossil and modern *Litsea*. PCA was performed using the stats package in R (R Core Team, 2012).

3. Results

3.1 Isotopes of leaf waxes and bulk sediment

Leaves and leaf waxes from the Foulden Maar Diatomite reveal clear changes in micromorphological properties and isotopic composition across the ~100 kyr interval of sediment deposition, and specifically contemporaneous with the termination of the Mi-1 glaciation (Fig. 4a). Leaf wax $\delta^{13}\text{C}_{\text{wax}}$ and bulk sediments ($\delta^{13}\text{C}_{\text{bulk}}$) decrease by 4–6 ‰ at ~23.01 Ma. Both $\delta^{13}\text{C}_{\text{wax}}$ and $\delta^{13}\text{C}_{\text{bulk}}$ increase back to their initial “pre-decline” value at ~22.99 Ma. Thus, the terrestrial carbon isotope record preserved in Foulden Maar sediments can be broadly divided into three Phases (Fig. 4). Phase 1 has relatively high $\delta^{13}\text{C}$ values (mean $\delta^{13}\text{C}_{\text{bulk}} = -28.8 \pm 1.1\text{‰}$, mean $\delta^{13}\text{C}_{\text{wt-avg}} = -29.5 \pm 1.5\text{‰}$); Phase 2 has relatively low $\delta^{13}\text{C}$ values (mean $\delta^{13}\text{C}_{\text{bulk}} = -31.8 \pm 1.1\text{‰}$, mean $\delta^{13}\text{C}_{\text{wt-avg}} = -32.4 \pm 2.3\text{‰}$); and Phase 3 has relatively high $\delta^{13}\text{C}$ values, similar to Phase 1 (mean $\delta^{13}\text{C}_{\text{bulk}} = -28.6 \pm 0.7\text{‰}$, mean $\delta^{13}\text{C}_{\text{wt-avg}} = -30.2 \pm 1.6\text{‰}$). Over the length of the core, individual $\delta^{13}\text{C}_{\text{wt-avg}}$ and $\delta^{13}\text{C}_{\text{bulk}}$ are correlated ($p < 0.01$) (Appendix B, Table B1).

Leaf wax δD values are negatively correlated to $\delta^{13}\text{C}$ values across the ~100 kyr period of sedimentation (Fig. 4b; Appendix B, Table B1). δD values are relatively low in Phase 1 (mean $\delta\text{D}_{\text{wax-C28}} = -136.6 \pm 13.0\text{‰}$, mean $\delta\text{D}_{\text{wax-C30}} = -158.5 \pm 4.0\text{‰}$, mean $\delta\text{D}_{\text{wax-C32}} = -159.2 \pm 2.4\text{‰}$), more positive in Phase 2, particularly in the 10 kyr interval where also the most notable shift in $\delta^{13}\text{C}_{\text{wax}}$ occurs (mean $\delta\text{D}_{\text{wax-C28}} = -125.6 \pm 9.1\text{‰}$, mean $\delta\text{D}_{\text{wax-C30}} = -148.0 \pm 12.1\text{‰}$, mean $\delta\text{D}_{\text{wax-C32}} = -150.9 \pm 7.9\text{‰}$) and return to relatively low values in Phase 3 (mean $\delta\text{D}_{\text{wax-C28}} = -139.2 \pm 10.4\text{‰}$, mean $\delta\text{D}_{\text{wax-C30}} = -154.8 \pm 4.6\text{‰}$, mean $\delta\text{D}_{\text{wax-C32}} = -154.9 \pm 3.8\text{‰}$).

3.2. Leaf properties in the Foulden Maar Diatomite

Stomatal density (SD) and stomatal index (SI) in fossil *Litsea calicarioides* leaves reveal large changes that are coincident with the three phases identified by the carbon isotope values (Fig. 4a). Leaves from Phase 1 have relatively high SD and SI ($SD = 189 \pm 43 \text{ mm}^{-2}$, $SI = 10.2 \pm 1.4$), leaves from Phase 2 have relatively low SD and SI ($SD = 140 \pm 70 \text{ mm}^{-2}$, $SI = 5.1 \pm 0.9$), and those from Phase 3 show a return to the high SD and SI values of Phase 1 ($SD = 243 \pm 67 \text{ mm}^{-2}$, $SI = 9.9 \pm 2.3$) (Appendix A, Table A6). Student's t-test for disparity reveals that mean SD and SI values in Phase 2 differ significantly ($p < 0.01$) from those of Phase 1 and Phase 3 (Appendix B, Table B2). We note that only one leaf sample (Sample 40_93_80_4) was recovered from the sediment interval corresponding to 22.99–22.97 Ma (the beginning of Phase 3); however, this leaf's SD and SI values are very similar to values from other Phase 3 leaves and suggests that the reversion of SD and SI values was coincident with the same transition in $\delta^{13}\text{C}$ values (Fig. 4a).

No marked changes were observed in aperture size (a_{max}), pore depth (p_{dpt}), or epidermal cell density (ED) of fossil *Litsea calicarioides* leaves across the ~100 kyr of sediment deposition at Foulden Maar (Phase 1: $a_{\text{max}} = 87.7 \pm 10.3 \text{ }\mu\text{m}^2$, $p_{\text{dpt}} = 5.1 \pm 0.4 \text{ }\mu\text{m}$, $ED = 1682 \pm 368 \text{ mm}^{-2}$; Phase 2: $a_{\text{max}} = 99.0 \pm 20.9 \text{ }\mu\text{m}^2$, $p_{\text{dpt}} = 4.8 \pm 0.6 \text{ }\mu\text{m}$, $ED = 2301 \pm 314 \text{ mm}^{-2}$; Phase 3: $a_{\text{max}} = 90.0 \pm 16.2 \text{ }\mu\text{m}^2$, $p_{\text{dpt}} = 5.0 \pm 0.4 \text{ }\mu\text{m}$, $ED = 2275 \pm 539 \text{ mm}^{-2}$) (Appendix A, Table A6). Student's t-test for disparity suggests ED, a_{max} and p_{dpt} during Phases 1, 2 and 3 do not differ significantly ($p > 0.05$), except Phase 1 ED differs from Phase 2 ($p < 0.05$) (Appendix B, Table B2).

3.3. Leaf properties and carbon isotopes in historic *Litsea calicaris herbaria* leaves

There is no trend in Δ ($R^2 = 0.115$, $p > 0.05$), $g_{c[\text{max}]}$ ($R^2 = 0.098$, $p > 0.05$), or SI ($R^2 = 0.007$, $p > 0.05$) of *Litsea calicaris* in response to last century C_a variation (Appendix A, Table A3, A4,

Appendix C, Fig. C2). However, there appears to be a weak, but statistically significant relationship between leaf mass per area and C_a ($R^2 = 0.188$, $p < 0.05$). $\delta^{13}C_{\text{leaf}}$ decreased during the last century, as expected in response to decreasing $\delta^{13}C_{\text{air}}$ (Appendix A, Table A3). Using a transformation of equation 2:

$$A_n = C_a \times \left(g_{c[\text{op}]} \times \left[1 - \frac{C_i}{C_a} \right] \right) \quad (3)$$

we calculate A_n of *L. calicaris* to be 2.5–5.9 $\mu\text{mol m}^{-2} \text{s}^{-1}$ ($\mu = 4.1 \pm 1.0 \mu\text{mol m}^{-2} \text{s}^{-1}$) over the last century.

Principal components analysis (PCA) on leaf micromorphological properties of *L. calicaris* and *L. calicarioides* reveals that approximately half of the variance (51%) between fossil and modern New Zealand *Litsea* is explained by larger a_{max} and p_{dpt} , and reduced ED in *L. calicaris* (Fig. 5). Variability in SD and SI correspond to the y-axis, explaining 29% of the variance (Fig. 5).

4. Discussion

4.1. Stomatal changes indicate pCO_2 increased at Mi-1 termination

The leaf micromorphology of *Litsea calicarioides* underwent a major transformation between Phase 1 and Phase 2 toward lower SD and SI (Fig. 4a), resulting in reduced $g_{c[\text{max}]}$. Reduction of leaf conductance has been interpreted as a long-term response of the species to an increase in C_a (e.g. Kürschner and Kvaček, 2009; Lammertsma et al., 2011). As C_a increases, diffusion through stomata increases leading to increased C_i and A_n (Fig. 3a) (Bowes, 1993). However, the response is non-linear because increased C_a allows the plant to reduce its susceptibility to water loss (Beerling and Woodward, 1995; Drake and González-Meler, 1997). This is achieved by reducing $g_{c[\text{max}]}$ to optimize the control of $g_{c[\text{op}]}$ to a potential water shortage (Franks et al., 2012). In other

words, the plant reduces the number of stomata through which it might lose water and optimizes $g_{c[op]}$ to most effectively respond to potential water shortages while retaining optimal photosynthetic rate.

Current theory concerning the leaf physiological response over millennial timescales supports our interpretation that the reduction of $g_{c[max]}$ from Phase 1 to Phase 2, and subsequent increase from Phase 2 to Phase 3 (Fig. 4a), reflects an increase in C_a during the ~20 kyr period comprising Phase 2 (Franks et al., 2012; 2014).

4.2 Isotopes reveal changes in hydroclimate

It has been proposed that $\delta^{13}C$ values of C3 plants is primarily controlled by atmospheric C_a (Feng and Epstein, 1995). However, while a C_a impact on $\delta^{13}C$ has been observed in short-term experimental conditions (e.g. Schubert and Jahren, 2012), in field studies, and over timescales of decades to millennia and longer, various studies have concluded that the impact of C_a on $\delta^{13}C$ values is negligible (Diefendorf et al., 2015; Kohn, 2016). In agreement with these latter studies, our investigation of historical (1900–2012) leaves indicates that the ~33% increase in pCO_2 during the 20th century (295–390 ppm) had a negligible effect on *L. calicaris* $\delta^{13}C$ values (Appendix C, Fig. C2).

L. calicaris is a rainforest component (de Lange, 2011) and its occurrence range in northern North Island, New Zealand very rarely experiences moisture deficits (Fig. 1b). It has been suggested that Δ of C3 plants from wet regions should primarily reflect C_a availability, because the impact of moisture variations on g_c is minimized in such plants (Schubert and Jahren, 2012). However, regardless of drought-tolerance, a rainforest tree is expected to increase its growth rate in response to precipitation changes if those precipitation changes are significant with respect to

its optimal climatic range (Fig. 3b). If precipitation changes result in greater optimization, then the plant will increase growth rate by increasing A_n , prompting a decrease in C_i and Δ (Fig. 3a). Our results indicate that within the past 100 years *L. calicaris* did not respond to the ~33% increase in C_a in a manner that resulted in decreased Δ .

In light of our results and those from other studies that fail to identify a direct impact of C_a on Δ , we do not interpret the large changes in $\delta^{13}C_{wt-avg}$ and $\delta^{13}C_{bulk}$ values in the Foulden Maar record as a response to C_a . Instead, we interpret the $\delta^{13}C$ changes to represent changes in hydroclimate (i.e. moisture availability) through time. This interpretation is consistent with results from meta-studies of modern plants (Diefendorf et al., 2010; Kohn, 2010) and is supported by the strong negative correlation between $\delta^{13}C$ and δD values (Fig. 4b; Appendix B, Table B1), which suggests that variations in transpiration rate (i.e. stomatal conductance) controlled both carbon and hydrogen isotope composition. We propose that at Foulden Maar, during intervals of reduced moisture availability plants restricted their transpiration rates, leading to less evaporative D-enrichment of leaf water and resulting in relatively low plant wax δD values. Reduced transpiration is necessarily associated with lower stomatal conductance and lower C_i , resulting in reduced Δ (i.e., higher $\delta^{13}C$ values). Within this framework, we interpret intervals of relatively high $\delta^{13}C$ values and low δD values (Fig. 4b, Phases 1 and 3) as periods of reduced transpiration in response to decreased water availability. During intervals with greater moisture availability the plants at Foulden Maar became less water conservative and transpiration rates increased, resulting in relatively high leaf water δD values and low $\delta^{13}C$ values (due to higher C_i and therefore increased Δ). The relationship we observe between $\delta^{13}C$ and δD values of leaf waxes in Foulden Maar sediments has also been observed among modern

C3 plant species, and has been attributed to differences in water conservation among different plant species (Hou et al., 2007).

This interpretation is inconsistent with the observations of Kahmen et al. (2013a,b), which indicate that in northern Australian *Acacia* and *Eucalyptus*, and in greenhouse experiments with a variety of plants, increased aridity leads to greater evaporative D-enrichment of leaf water and an *increase* in leaf wax δD values. These observations imply that the plants studied by Kahmen et al (2013a,b) were unable to effectively restrict transpiration to fully counteract the increased leaf water evaporation caused by enhanced aridity. However, the transect (Kahmen et al., 2013a) and greenhouse studies (Kahmen et al., 2013b) are not directly analogous to our study for a number of reasons that we believe can explain this apparent discrepancy. Firstly, the leaf waxes in our study represent gradual ecosystem response to environmental changes that took place over long timescales (100,000 years). The response would likely have included genotypic trait evolution, and was not limited to plastic responses. Secondly, the studies of Kahmen et al (2013a,b) focus on open forest or scrubland plants in a highly monsoonal to arid environment, whereas the paleoenvironment of Foulden Maar was neither monsoonal nor arid (growth season precipitation ~2200 mm, dry season precipitation ~280 mm; Reichgelt et al., 2013). Instead, similar to the modern environment at Blood Pond, Massachusetts (Hou et al., 2007), the Foulden Maar depositional environment would have had annual net moisture excess, rather than extreme moisture deficit. Kahmen et al. (2013a) report that different plants in their study expressed very different magnitudes of leaf water D-enrichment, possibly due to differences in their ability to counteract aridity changes with changes in transpiration rate. Perhaps plants that are operating near the limits of their water conservation potential, such as plants that grow in arid environments, do not have much additional capacity to reduce their transpiration rate in response

to increasing aridity. Such plants would exhibit a large δD response to aridity change, due to enhanced evaporative D-enrichment of leaf water. Conversely, plants with the capacity to reduce their transpiration rates in response to reduced moisture availability would have a smaller (or even a reversed) δD response to changes in moisture availability. Thus, while we interpret the leaf wax δD record from Foulden Maar to reflect hydroclimate variability, the leaf wax δD variations most likely do not reflect the same mechanistic response to aridity observed by Kahmen et al. (2013a,b). Instead, at Foulden Maar relatively high $\delta^{13}C$ values together with relatively low δD values reflect periods of reduced moisture availability. The three different phases identified by the $\delta^{13}C$ values correspond to the largest hydroclimate shifts. Phase 2 had substantially more negative $\delta^{13}C$ values and more positive δD values than Phase 1 and 3, and therefore represents a period of increased transpiration related to increased moisture availability.

Notably, during Phase 2, while $\delta^{13}C$ and δD values indicate increased moisture supply to the ecosystem, leaves of *L. calicarioides* reveal *reduced* $g_{c[max]}$. This could indicate that increased moisture availability in Phase 2 directly resulted in a decrease in $g_{c[max]}$ in *L. calicarioides*. However, this is unreasonable because increased moisture availability is expected to lead to an *increase* in $g_{c[max]}$, because with reduced water stress a plant can become less water-conservative and better optimize carbon uptake (Beerling and Woodward, 1995; Drake and González-Meler, 1997). Therefore, we infer that while $\delta^{13}C$ and δD values of leaf material were primarily influenced by moisture availability, the leaf micromorphological structures (i.e. SD , $g_{c[max]}$) were responding to changes in pCO_2 .

4.3 Implications for pCO_2

To quantify $p\text{CO}_2$ changes, we iteratively solved for $g_{c[\text{op}]}$, A_n , and C_a of early Miocene *Litsea calicarioides* based on leaf micromorphological properties (Appendix A, Table A6), $\delta^{13}\text{C}$ values (Appendix A, Table A7), and the modern relationship between A_n and C_a in *L. calicaris* (Appendix A, Table A3) using the Franks et al. (2014) model (Appendix A, Table A5). The model provides (Appendix A, Table A8) similar C_a estimates during Phase 1 and Phase 3 (516 ± 111 ppm and 425 ± 53 ppm, respectively), with consistent convergence ($P_{\text{max}} = 24.8 \pm 4.6\%$ and $23.4 \pm 3.8\%$) (Appendix C, Fig. C3, C4). This estimate is in broad agreement with other early Miocene $p\text{CO}_2$ estimates (Zhang et al., 2013). C_a estimates for Phase 2 are much higher (1144 ± 410 ppm), but the certainty of this convergence is relatively low ($P_{\text{max}} = 6.1 \pm 3.4\%$). The low convergence in Phase 2 is the result of the large range of possible C_a solutions when $g_{c[\text{op}]}$ is low, C_i/C_a is high, and A_n varies within the same range of solutions.

Mean C_a estimates for Phase 2 are more than double the estimates for Phase 1 and 3. However, even the lowermost end of the 2σ uncertainty range for Phase 2 C_a estimates (~ 744 ppm) exceeds the threshold atmospheric CO_2 levels required for the termination of the Mi-1 event (DeConto et al., 2008; Wilson et al., 2008). During Quaternary glaciations, C_a variability between glacial and interglacial times are primarily thought to have been the result of CO_2 transfer between the deep ocean and atmosphere (Anderson et al., 2009). The degree of ventilation of deep water in the Southern Ocean is believed to provide the major control on the release of CO_2 from the ocean into the atmosphere, and the same mechanism could be responsible for the observed C_a changes inferred from the Foulden Maar record (Mawbey and Lear, 2013). However, the magnitude of Quaternary $p\text{CO}_2$ changes were on the order of 100 ppm (180–280 ppm) between glacial and interglacial states, while our estimates imply an increase on the order of ~ 500 ppm (500–1000 ppm) near the O/M boundary. This would either require much

greater release of deep ocean CO₂ at the Oligocene/Miocene boundary than occurred at the onset of Quaternary interglacials, or another unidentified reservoir (or multiple reservoirs) of exchangeable CO₂. Potential sources of CO₂ could include enhanced organic carbon oxidation in the Southern Ocean due to southward migration of the Westerly Wind belt (Mawbey and Lear, 2013) or enhanced feedback mechanisms such as chemical weathering (Lear et al., 2004). The rapid timing (~20 kyr) of the increase in atmospheric carbon is not unprecedented (McInerney and Wing, 2011); however there is no other proxy evidence that indicates increased atmospheric CO₂ at the Oligocene/Miocene boundary (Zhang et al., 2013).

An atmospheric *p*CO₂ rise on the order of 500 ppm is expected to result in a large global temperature rise (Hansen et al., 2013). Indeed, there is some evidence for substantial temperature rise at the Oligocene/Miocene boundary, inferred from Mg/Ca and δ¹⁸O values in deep-water foraminifera (Lear et al., 2004; Pälike et al., 2006; Liebrand et al., 2011), which may be related to changes in atmospheric CO₂. However, due to the chronological uncertainties involved, it is not possible to say that the marine temperature changes relate to the *p*CO₂ changes inferred from Foulton Maar. Mildenhall et al. (2014) demonstrated that little vegetation change is found in the downcore sediments from Foulton Maar, suggesting that the temperature/precipitation effect had little effect on the regional vegetation. However, the Foulton Maar pollen assemblages were strongly overprinted by wind-blown *Nothofagus* pollen (Mildenhall et al., 2014), which may obscure a local signal of vegetation change. Therefore, at present there are insufficient data to evaluate the potential impact of our inferred *p*CO₂ increase on global temperature.

4.4. Modern vs. Miocene Litsea

Anthropogenic carbon emissions have increased C_a levels by more than 30% since 1900. Plant physiological changes have been reported in response to this increase, including reduced conductance (e.g. Woodward, 1987; Lammertsma et al., 2011), increased Δ (e.g. Schubert and Jähren, 2012), and increased LMA (Poorter et al., 2009). In *Litsea calicaris* we observe a statistically significant change in LMA during the past 100 years, whereas Δ and $g_{c[\max]}$ appear unresponsive (Appendix C, Fig. C2). This implies that for *L. calicaris* the increase in C_a from 300 to 400 ppm during the past ~100 yrs did not lead to a change in Δ and $g_{c[\max]}$. However, the slight increase in LMA suggests that *L. calicaris* has been assimilating carbon more efficiently as C_a has increased. The absence of a *L. calicaris* SI response to a 100ppm increase in C_a during the past 100 years (Appendix A, Table A4) makes it impossible to use a SI regression function to estimate C_a , as other researchers have (Woodward, 1987; Kürschner et al., 1997). Furthermore, the model of Franks et al. (2014) relies on variation in fractionation and conductance to converge on a most likely C_a . Therefore, in the case of *L. calicaris*, the model is unable to discern the effects of variation in other environmental variables from changes in C_a within the 300 to 400 ppm range.

At first glance, these results seem to conflict with our interpretation that fossil leaves in the Foulden Maar sediments record changes in C_a . However, the anthropogenic C_a rise of the recent past does not provide a good analogy to the Foulden Maar record, due to differences in timescale. The recent C_a rise has taken place over a very brief time period, certainly shorter than the time necessary for the number of generational turnovers required to transform non-plastic plant morphological traits. We expect *L. calicaris* to eventually express the predicted reduction in $g_{c[\max]}$, but only after a sufficient number of generational turnovers has occurred. In contrast, the changes we observe at Foulden Maar took place over much longer timescales (centuries to

millennia) and therefore document physiological transformations occurring across many *L. calicarioides* generations. Plants optimize their phenotype in response to environmental signals received by previous leaf generations (Šantrůček et al., 2014). Genotypic optimization to environmental change happens over many generations (Franks et al., 2012), because next-generation cotyledons with a genotype optimal for the environment are the most likely to grow to adult trees and pass their genes to the subsequent generation. However, for trees such as *L. calicaris* with a century-long life span, this selective process is likely negligible over the course of only a century. Instead, we expect changes in plastic leaf phenotypes, which respond across leaf generations, to be most variable across a single century. On the other hand, the ~100 kyr record of *L. calicarioides* probably comprises 600–1000 generations and therefore represents “non-plastic” leaf transformations that occur across many plant generations.

Another possibility for the apparent unresponsiveness during the past century is that within the range of 300–400 ppm, environmental factors other than C_a are more important in determining Δ and $g_{c[\max]}$ in *L. calicaris*. Perhaps a 30% C_a increase within a century at ambient C_a of 400–500 ppm (i.e. early Miocene estimates from our and other data; e.g. Zhang et al., 2013) would yield a larger response in *L. calicaris*. Lammertsma et al. (2011) tested the response of $g_{c[\max]}$ in nine phylogenetically disparate Florida plant species to a 280–400 ppm increase in C_a and found that $g_{c[\max]}$ sensitivity differed widely, even among closely related species. They emphasize the difference in physiological response to C_a and the potential of different species to depend more strongly on variation in other environmental factors. The sensitivity of SI (as a $g_{c[\max]}$ proxy) to C_a in other species of Lauraceae has also been widely disparate (Greenwood et al., 2003; Kürschner and Kvaček, 2009; Barclay et al., 2010). Thus, we cannot exclude the

possibility that the sensitivity of $g_{c[\max]}$ in *L. calicaris* to C_a is below a certain threshold value, and that its response can therefore not be detected within the 300–400 ppm range.

Finally, despite strong morphological similarities, *L. calicaris* and *L. calicarioides* are separated by 23 million years of evolution and environmental change and might therefore respond differently to environmental variation. Modern *L. calicaris* has larger $a_{\max}$, greater p_{dpt} , and lower ED than the *L. calicarioides* fossils (Fig. 5). These traits are considered environmentally plastic across leaf generations (within plant generations; Šantrůček et al., 2014) and the differences could be due in part to the fact that *L. calicaris* grows today in warm-temperate climates and the *L. calicarioides* at Foulden Maar grew in a subtropical environment (Reichgelt et al., 2013). The differences in $a_{\max}$ and p_{dpt} between *L. calicaris* and *L. calicarioides* could also potentially reflect the presence of a larger genome size in *L. calicaris* (Beaulieu et al., 2008). Thus, there are a number of reasons to anticipate that leaf micromorphological parameters in *Litsea* (as well as other long-lived plants) would respond differently to changes in $p\text{CO}_2$ depending on the timescales involved.

4.5. The role of local ecology

Modern-day *L. calicaris* is generally considered a minor subcanopy component in New Zealand's North Island rainforests, but is dominant when competition is low (de Lange, 2011). The position a leaf occupies within the vertical structure of a rainforest can impact water-use efficiency and carbon uptake, due to different irradiance levels and the utilization of respired CO_2 (Graham et al., 2014). Ecological succession, driven for example by rainfall changes, may have resulted in periods during which canopy competition for light was greater. If A_n decreased in response to reduced irradiance levels, plants would be able to become less efficient in their

water usage, resulting in increased ζ and δD values. Simultaneously, C_i and Δ would increase and A_n would be relatively low. If *L. calicarioides* became more shaded during this period as it became part of the subcanopy, it could have decreased $g_{c[\max]}$ (Šantrůček et al., 2014).

The scenario of local ecology driving physiological change is plausible; however, there are several key factors why this scenario is less likely than the case for changes in pCO_2 and rainfall as drivers of variation in $g_{c[\max]}$, $\delta^{13}C$ and δD . Importantly, the timing of decrease in SI differs from the peak in the $\delta^{13}C$ and δD of the C32 fatty acid by ~10 kyr (Fig. 4a,b). This suggests that leaf morphology changed prior to the decrease in water-use efficiency. Additionally, typically when A_n drops and C_i increases, leaves produce relatively larger epidermal cells, thereby decreasing ED (Šantrůček et al., 2014). In the Foulden Maar record of *L. calicaroides* there is no indication of significant changes in ED concomitant with changes in SD and SI (Appendix B, Table B2).

5. Conclusion: leaf physiological change at the termination of the Mi-1

We present a ~100 kyr record of leaf micromorphological change in *Litsea calicarioides* (Lauraceae), and leaf wax $\delta^{13}C$ and δD values from southern New Zealand spanning the termination of the Mi-1 event. The records reveal three distinct phases during the 100 kyr interval. Phase 1 (23.03–23.01 Ma) and Phase 3 (22.99–22.94 Ma) have high stomatal conductance, relatively high $\delta^{13}C$ values, and relatively low δD values, a combination that we interpret to reflect increased transpiration in the ecosystem (Fig. 4b). By contrast, Phase 2 (23.01–22.99 Ma) is characterized by low conductance, relatively low $\delta^{13}C$ values, and relatively high δD values, a combination that we interpret to reflect reduced transpiration in the ecosystem. Together, $\delta^{13}C$ and δD values of leaf waxes (Fig. 4b) reveal a period of increased ecosystem

transpiration during Phase 2. We interpret this as an increase in local precipitation during that period.

The 20 kyr period of Phase 2 appears to coincide with the onset of glacial termination out of the Mi-1 event. The abrupt decrease in stomatal conductance likely represents an increase of atmospheric CO₂ (C_a) associated with the glacial termination. Using the leaf gas exchange model of Franks et al. (2014), we estimate that ambient levels of C_a were 516 ± 111 ppm and 425 ± 53 during Phases 1 and 3, respectively, in agreement with other proxy records (e.g. Zhang et al., 2013). We estimate that during Phase 2 C_a was 1.5–3× higher (1144 ± 410 ppm) and therefore sufficiently high to initiate the Antarctic deglaciation out of Mi-1 (DeConto and Pollard, 2003; DeConto et al., 2008).

The closest living relative of *L. calicarioides*, *L. calicaris*, did not significantly alter g_{c[max]} or Δ, but did increase leaf mass per area (LMA), between 1900–2012 A.D., during which time C_a increased from 295 to 390 ppm (Appendix C, Fig. C2). This suggests that *L. calicaris* increased carbon assimilation efficiency, but that the increase in C_a was either below the sensitivity threshold needed to instigate a phenotypic change, or that the ~100 year interval was too brief to allow the number of generational turnovers needed for the New Zealand population of *L. calicaris* to respond to a C_a rise. Thus, we underscore the important point that the timescale available for plant response is an important consideration when using plastic traits to evaluate environmental change.

Acknowledgements

Wei Huang, Andy Juhl, Betina Fleming and Nicole deRoberts are acknowledged for technical support. Jennifer Bannister is gratefully acknowledged for her taxonomic identifications.

Dhahara Ranatunga and Ewen Cameron of the Auckland War Memorial Museum Herbarium are acknowledged for supplying modern material. This work was funded by NSF-EAR13-49659 and a Vetlesen Foundation grant to WJD. The Center for Climate and Life of Columbia University provided funding for technician support. We gratefully acknowledge two anonymous reviewers for their insightful comments on this manuscript. We extend our gratitude towards Daphne Lee for her excellent coordination of research at Foulden Maar.

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Figure captions

Fig. 1a. Live foliage of *Litsea calicaris* (mangeao). **b** Map of New Zealand with locations of modern *L. calicaris* samples, superimposed on a January vapor pressure deficit map from LENZ (2012). Note that the three samples north of the northern tip of the main New Zealand landmass are from the remote Three Kings Islands. **c** Map of New Zealand with range of modern *L. calicaris* indicated in green and location of Foulden Maar (black circle).

Fig. 2a. Leaf of *Litsea calicaris* (specimen AK162971); scale is 10 mm. **b** Leaf fossil of *Litsea calicarioides* (specimen OU33374); scale is 10 mm. **c** Leaf cuticle of *L. calicaris* (specimen AK99642a); scale is 100 µm, including inset of individual pore, with arrows to 1) polar rods, 2) peristomatal rim and 3) narrow, indistinct stomatal scales. **d** Leaf cuticle of *L. calicarioides* (specimen 23n); scale is 100 µm, including inset of individual pore, with same indications as **c**.

Fig. 3a. Schematic overview of feedback mechanisms operating in leaf carbon assimilation (A_n), red arrows indicate positive feedbacks and blue negative feedbacks, including maximum aperture size (a_{max}), pore depth (p_{dpt}), stomatal density (SD), maximum conductance ($g_{c[max]}$), mesophyll conductance (g_m), operational conductance ($g_{c[op]}$), stomatal opening time fraction (ζ), carbon

fractionation (Δ), intercellular carbon (C_i), pavement cell density (ED), and leaf mass per area (LMA). **b** Schematic visualization of A_n optimization in response to the environment, red arrows indicating change towards the optimal range of the climatic envelope and the blue arrows towards the suboptimal range of the climatic envelope. **c** Schematic visualization of A_n optimization in response to changes in atmospheric carbon (C_a), here too, the red arrow indicates change towards the optimal range of A_n in response to C_a and the blue arrow towards the suboptimal range.

Fig. 4a. Summary of micromorphological and $\delta^{13}\text{C}$ changes in the Foulden Maar sediments. Grey circles indicate individual SI measurements and black circles with error bars the mean and standard deviations of SI. The red line represents the progression of $\delta^{13}\text{C}_{\text{bulk}}$ from Foulden Maar and the blue line the progression of $\delta^{13}\text{C}$ of C28, C30 and C32-fatty acids ($\delta^{13}\text{C}_{\text{wt-avg}}$). The yellow area indicates the period (Phase 2) during which the fractionation (Δ) at Foulden Maar was high, while the stomatal index (SI) of *Litsea calicarioides* was low. **b** Covariance of $\delta\text{D}_{\text{wax-C32}}$ and $\delta^{13}\text{C}_{\text{wax-C32}}$ in the Foulden Maar core. Increased water use efficiency and decreased transpiration is indicative of drier conditions.

Fig. 5. Results of principal component analyses (PCA). Modern *Litsea calicaris* leaf micromorphological properties analyzed together with leaf micromorphological properties of fossil *L. calicarioides* from Foulden Maar.

Figure 2

Figure 3

Figure 4

Figure 5

Table 1. List of abbreviations and terms commonly used in this paper.

Abbr.	Parameter	Description
C_a	Atmospheric pCO_2	Amount of atmospheric CO_2 particles per million particles
C_i	Intercellular pCO_2	Amount of CO_2 particles per million particles in leaf intercellular space
$\delta^{13}C$	Carbon isotope ratio	$^{13}C/^{12}C$ in ‰, relative to VPDB
δD	Hydrogen isotope ratio	$^2H/^1H$ in ‰, relative to VSMOW
Δ	Fractionation rate	Difference between $\delta^{13}C$ of source and endmember because of discrimination in biological transfer of carbon
A_n	Assimilation/ Photosynthetic rate	Rate at which carbon is fixed during carboxylation, in μmol of carbon per m^2 of leaf per second
$g_{c[op]}$	Operational conductance	Total amount of CO_2 that is diffused from the atmosphere to the leaf intercellular space, in mol of carbon per m^2 of leaf per second
$g_{c[max]}$	Maximum conductance	Maximum amount of CO_2 that could be diffused through the leaf stomata, in $mol\ m^{-2}\ s^{-1}$
g_b	Boundary layer conductance	Amount of CO_2 that is diffused through the air space outside of the leaf, but influenced by leaf physiology, in $mol\ m^{-2}\ s^{-1}$
g_m	Mesophyll conductance	Amount of CO_2 that is diffused through the leaf mesophyll, in $mol\ m^{-2}\ s^{-1}$
SD	Stomatal density	Number of stomata (pores) on the leaf per unit area
ED	Epidermal cell density	Number of epidermal cells on the leaf per unit area
SI	Stomatal index	Ratio of stomata relative to the total number of cell on the leaf
a_{max}	Maximum aperture size	Maximum area of the pore opening
p_{dpt}	Pore depth	Depth of the pore opening
LMA	Leaf mass per area	Leaf organic mass per unit area
ζ	Stomatal opening fraction	Amount of time stomata were open divided by total time passed