Accepted Manuscript

Significant transient pCO2 perturbation at the New Zealand Oligocene-Miocene transition recorded by fossil plant stomata

Margret Steinthorsdottir, Vivi Vajda, Mike Pole

PII: S0031-0182(17)30733-2
DOI: https://doi.org/10.1016/j.palaeo.2018.01.039
Reference: PALAEO 8648
To appear in: Palaeogeography, Palaeoclimatology, Palaeoecology

Received date: 6 July 2017
Revised date: 21 January 2018
Accepted date: 27 January 2018

Please cite this article as: Margret Steinthorsdottir, Vivi Vajda, Mike Pole, Significant transient pCO2 perturbation at the New Zealand Oligocene-Miocene transition recorded by fossil plant stomata. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Palaeo(2017), https://doi.org/10.1016/j.palaeo.2018.01.039

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Significant transient $p$CO$_2$ perturbation at the New Zealand Oligocene-Miocene transition recorded by fossil plant stomata

Margret Steinthorsdottir$^{a,b,*}$, Vivi Vajda$^a$, Mike Pole$^c$

$^a$Department of Palaeobiology, Swedish Museum of Natural History, SE 104 05 Stockholm, Sweden; $^b$Bolin Centre for Climate Research, Stockholm University, SE 109 61 Stockholm, Sweden; $^c$Queensland Herbarium, Brisbane Botanic Gardens, Mt. Coot-tha, Mt. Coot-tha Rd., Toowong, QLD 4066, Australia.

*Corresponding author: margret.steinthorsdottir@nrm.se
Abstract

The reorganisation of Earth’s climate system from the Oligocene to the Miocene was influenced by complex interactions between Tethyan tectonics, orbital parameters, oceanographic changes, and carbon cycle feedbacks, with climate modelling indicating that $pCO_2$ was an important factor. Oscillating episodes of climate change during the Oligocene–Miocene transition (OMT) have however been difficult to reconcile with existing $pCO_2$ records. Here we present a new $pCO_2$ record from the OMT into the early Miocene, reconstructed using the stomatal proxy method with a database of fossil Lauraceae leaves from New Zealand. The leaf database derives from three relatively well-dated sites located in the South Island of New Zealand; Foulden Maar, Mataura River and Grey Lake. Atmospheric $pCO_2$ values were obtained based on four separate calibrations with three nearest living equivalents, using the stomatal ratio method as well as transfer functions. Our results, based on the mean values of each of the four calibrations, indicate $pCO_2$ ranging ~582–732 ppm (average 650 ppm) during the OMT, falling precipitously to mean values of ~430–538 ppm (average 492 ppm) for the earliest Miocene and ~454–542 ppm (average 502 ppm) in the early Miocene. The much higher values of $pCO_2$ at the OMT indicate that $pCO_2$ played an important role in climate dynamics during this time, potentially including the abrupt termination of glaciations.

Keywords: stomatal proxy; climate change; Oligocene–Miocene boundary; Mi-1 termination; Lauraceae
1. Introduction

1.1. The Oligocene–Miocene boundary and climate transition

The Oligocene–Miocene boundary (OMB) is dated at 23.03 million years ago (Ma) in the ICS International Chronostratigraphic Chart (Cohen et al., 2016), based on interpretations of distinct obliquity patterns in marine pelagic sediments, although without preserved magnetostratigraphy (Pälike et al., 2006; Shackleton et al., 2000). The OMB marks the important climate transition from the still relatively warm Paleogene to the cooler Neogene, but in contrast to e.g. the Eocene–Oligocene boundary (see Coxall et al., 2005), the OMB is not easily recognizable globally and must be defined at regional boundaries. The New Zealand stage system, and its correlation with the international system, is documented in Cooper (2004) and updated by Raine et al. (2015). New Zealand's late Oligocene–Miocene palynological zones typically have wide age estimates, due to lack of sufficient marine control. As one consequence, the international stratigraphic OMB is not identified in the New Zealand stratigraphy, but rather contained within the Waitakian stage, which spans 25.2–21.7 Ma.

The Oligocene–Miocene climate transition (OMT) was a transient global cooling event, culminating in significant Antarctic ice sheet expansion, global sea-level fall and a cooling of 2 °C or more (Miller et al., 1991; Liebrand et al., 2011; Mawbey and Lear, 2013; Sliwinska et al., 2014; Liebrand et al., 2017). Peak glaciation as interpreted from marine oxygen isotope records – the Miocene isotope zone 1 glaciation (Mi-1: as defined by Pälike et al. (2006)) – took place just prior to and at the OMB, within the OMT, followed by ice sheet retreat/warming of similar magnitude (Zachos et al., 2001b; Lear et al., 2004; Larsson et al., 2006; Pälike et al., 2006; Wilson et al., 2008; Larsson et al., 2011; Mawbey and Lear, 2013).
The Mi-1 took place in two “steps” marked by maximum benthic foraminifera δ\textsuperscript{18}O excursions, each episode lasting 200–300 kyr (Lear et al., 2004; Liebrand et al., 2011), and was a part of a cyclic oscillation pattern of glaciation/deglaciation during the Oligocene to early Miocene (Liebrand et al., 2017). The deglaciation phase at the end of Mi-1 is marked by accelerated sedimentation rates, interpreted to represent increased biogenic productivity (Florindo et al., 2015). Recent results indicate that although multiple marine isotope proxy records independently support the overall OMT trends, significant inter- and intrabasinal variability in deep water masses exists, together with chronological discrepancies, complicating interpretations of this global event (Florindo et al., 2015; Beddow et al., 2016; and adding further to the inherent limitations of the marine isotope proxy records, which record both temperatures and ice sheet volumes, in unknown proportions (Wilson et al., 2008).

Terrestrial vegetation records confirm a global cooling episode associated with Mi-1 and a subsequent significant transient warming (Kürschner and K vakcek, 2009; Larsson et al., 2010; Kotthoff et al., 2014). In New Zealand, \textit{Nothofagus} pollen was usually dominant through the Oligocene and into the early Miocene, following which there was a marked increase in palynological diversity, with a variety of dominant taxa. This is interpreted to reflect cooler followed by warmer climatic conditions (Mildenhall, 1980; Pocknall, 1982a; Pocknall and Mildenhall, 1984; Mildenhall and Pocknall, 1989; Morgans et al., 2004).

1.2. The role of pCO\textsubscript{2} as forcing mechanism at the Mi-1 termination

The forcing and feedback mechanisms involved in the initiation and termination of OMT glaciations, including the Mi-1, are still not fully understood, with orbital parameters, ocean circulation changes due to closing of the Tethys seaway and other gateway dynamics,
decreasing atmospheric CO$_2$ concentration ($p$CO$_2$) and the interplay with climatic thresholds all having been implicated in various combinations and proportions (e.g. Kennett and Shackleton, 1976; Miller et al., 1991; Paul et al., 2000; Zachos et al., 2001a,b; DeConto and Pollard, 2003; Lear et al., 2004; DeConto et al., 2008; von der Heydt and Dijkstra, 2008; Wilson et al., 2008; Mawbey and Lear, 2013; Zhang et al., 2013; Liebrand et al., 2017). During most of the Cenozoic, complex tectonic activity was ubiquitous, including e.g. building of the Rocky Mountains in USA as well as the Pyrenees and the main Alpine orogeny in Europe, north of Tethys. The Atlas Mountains in North Africa formed south of Tethys, and major orogenic activity along the Indonesia-Malaysia-Japan island arc system took place. India collided with the Asian continent, resulting in uplift of the Tibetan Plateau and the establishment of the Himalayan Mountains (Rosenbaum and Lister, 2002; Qin et al., 2017). A transgression that was initiated during the Late Cretaceous peaked around the latest Oligocene/earliest Miocene and much of the New Zealand subcontinent was submerged (Landis et al., 2008). A regression commenced soon after, but major tectonic activity and mountain building (the Kaikoura Orogeny, resulting eventually in the Southern Alps) did not occur until late Miocene–Pliocene (Youngson et al. 1998; King, 2000; Mortimer et al. 2001). The closing of Tethys thus led to the formation of numerous mountain ranges, resulting in increased weathering and draw-down of CO$_2$, causing an overall global cooling. This tectonic phase also included subduction of carbonate-rich oceanic crust and periodic volcanic activity, adding CO$_2$ to the atmosphere and during some intervals causing transient episodes of global warming (DeConto and Pollard, 2003; Edmond and Huh, 2003, Lyle et al., 2008).

Ice sheet modelling indicates that large parts of the Antarctic ice sheet, once formed, should have remained relatively stable (hysteresis effect) and thus have not been able to account for the episodes of ice sheet advance and retreat – strongly indicated by geological evidence – under stable, moderate $p$CO$_2$ forcing (Naish et al., 2001; Mawbey and Lear, 2013;
Carbon cycle feedbacks have been implicated as an important factor for the OMT transient cooling and subsequent warming, through first enhanced organic carbon burial as a positive feedback to cooling climate (Mawbey and Lear, 2013; Florindo et al., 2015), followed by increased greenhouse forcing due to decrease in chemical weathering rates during glaciation (Lear et al., 2004), or organic matter oxidation (Mawbey and Lear, 2013), a negative feedback. A seafloor carbonate dissolution event has been interpreted to reflect an input of carbon into the ocean-atmosphere system during the deglaciation phase of the OMT (Mawbey and Lear, 2013). The abrupt warming and deglaciation at the end of Mi-1 is linked to these dynamics, with a considerable increase in productivity observed shortly after the $\delta^{18}O$ maximum (ca. 100 kyr), signalling reduced carbon burial in the oceans and rapidly increasing $pCO_2$ (Diester-Haass et al., 2011; Florindo et al., 2015), independently supported by dissolution events at deep water sites (Mawbey and Lear, 2013). Few modelling studies exploring the relationship between $pCO_2$ and ice sheet growth at the OMT and Mi-1 have so far been published (Liebrand et al., 2011), but studies focusing on the previous rapid cooling and Antarctic glaciation at the Eocene–Oligocene boundary indicate that $pCO_2$ played an important role in the initiation of glaciation, and that orbital forcing also has a strong influence on ice sheet growth – especially near the glaciation threshold (DeConto and Pollard, 2003; Steinthorsdottir et al., 2016a). The glaciation threshold of $pCO_2$ for Antarctic glaciation however, historically assumed to be ca. 700–800 ppm, has recently been shown to be highly dependent on the type of climate model used and assumptions made (Gasson et al., 2014; 2016), fully re-opening the important question of the Earth system’s climate sensitivity to changing $pCO_2$.

In order to illuminate the complex respective roles of carbon cycle dynamics vs. orbital and/or tectonic and oceanographic factors in the abrupt climate transitions during OMT,
multiple independent records of $pCO_2$ are needed. This will allow both inter-comparison between $pCO_2$ records for maximum fidelity, and comparison and reconciliation with isotope-based temperature and other geological records. Existing marine proxy-derived $pCO_2$ estimates across the OMT indicate an overall trend of decreasing $pCO_2$, from ~500 ppm during the late Oligocene to “near modern values” (now below modern values) of ~370–380 ppm at the OMT (Pearson and Palmer, 2000; Pagani et al., 2005; Zhang et al., 2013) (Fig. 1). The warming episode terminating the Mi-1 was not detected as a parallel feature in the marine $pCO_2$ records, leading to the assumption that $pCO_2$ was not a primary driver of climate and ice-sheet development during OMT (Pagani et al., 2005; Zhang et al., 2013). Very few previously published terrestrial stomatal proxy-based $pCO_2$ records exist for the late Oligocene and early Miocene, including across the transitional interval, but those that do generally converge with the marine-derived estimates at ~500–600 ppm for the latest Oligocene and ~400–500 ppm for the early Miocene (Kürschner et al. 2008; Beerling and Royer, 2011; Foster et al., 2012; Grein et al., 2013; Zhang et al., 2013; Roth-Nebelsick et al., 2014; Steinthorsdottir et al., 2016a) (Fig. 1). However, the stomatal proxy records listed above have all been of fairly low resolution, with either no or few data points at the OMT, and thus liable to miss any rapid transient fluctuations in $pCO_2$. Recently, Reichgelt et al. (2016) published an extraordinarily high-resolution record of leaf physiological change focused on the OMT (~23.02–22.94 Ma) derived from the Foulden Maar locality, New Zealand, indicating that $pCO_2$ may have doubled during the termination of Mi-1, from ~500 ppm briefly to ~1100 ppm (Fig. 1), before decreasing again to ~425 ppm. These results thus invite renewed discussions regarding the role of $pCO_2$ in the OMT climate dynamics.

Here we present a new stomatal proxy-based $pCO_2$ record using a database of fossil Lauraceae specimens from three localities across the OMT of New Zealand. We compare the
results with previously published $p$CO$_2$ records and discuss the implications for climate dynamics across the OMT, particularly the role of $p$CO$_2$ in terminating the Mi-1 glaciation.

2. Material and methods

2.1. Geological setting

The fossil Lauraceae leaves studied here were collected for previous taxonomic studies (Pole, 1993a; 2007; Pole et al., 2003) and derive from three outcrop successions in New Zealand’s South Island: 1) Diatomite sediments from outcrops at Foulden Maar, dated to 23.2 ± 0.2 Ma (Lindqvist and Lee, 2009), i.e. within the Watakian stage and close to the International OMB; 2) Mataura River sediments dated to earliest Miocene (late Watakian); and 3) the Grey Lake locality (informal local name) dated to early Miocene (Otaian) (Fig. 2).

The Foulden Maar fossil lake contains a high-resolution sedimentary succession of laminated diatomite, with abundant well-preserved fossil plants, first documented by Cecilia Travis in an unpublished thesis and subsequently described in detail and published by Pole (1993a; 1996: New Zealand Geological Society Fossil Record Number I43/f8503); Bannister et al. (2012); Conran et al. (2013) and Reichgelt et al. (2013). Radiometric dating of associated basalts constrains the Foulden Maar as 23.2 ± 0.2 Ma (Lindqvist and Lee, 2009), here referred to as the OMT.

At Mataura River, the so-called Gore Lignite Measures are exposed in several locations along the banks of the Mataura River, between the towns of Mataura and Gore in Southland (Fig. 2). An exposure in the bank of the Mataura River, within a few meters of the M1 coal seam, was placed in the lower half of the Rhoipites waimumuensis pollen Zone by Pocknall...
(in Isaac et al. 1990), classifying it as one of the most basal occurrences of the Gore Lignite Measures. This was probably the same as Couper’s (1960) S178/513 locality, regarded as Waitakian based on the predominance of *Nothofagidites matauraensis* and *N. cranwellae* pollen, but rarity of *Nothofagus “fusca”* type and Podocarpaceae pollen. The sediments are rich in leaf material, although not always well preserved. The *R. waimumuensis* Zone was regarded as mostly late Oligocene but extending into the early Miocene by Pocknall and Mildenhall (1984). They showed the marine sediments at the base of drillhole d1134 as being within the zone, and this was subsequently dated as basal Altonian by Pocknall (1982b). However, Crundwell et al. (2004) place the *R. waimumuensis* Zone in the Waitakian Stage, with its lower zone limit at the International OMB, thus the Mataura River locality may be regarded as ~23 ± 1 Ma (Waitakian, earliest Miocene age), and therefore largely synchronous with Foulden Maar. Here, we plot the results from Matauara River sediments at 22.5 Ma, which is the mid-point between the lower and upper limits of the age estimates.

Grey Lake is a local name for an abandoned sluice-gold-mining area near St Bathans (Fig. 2). It contains extensive outcrops of the St Bathans Member (Dunstan Formation), the lowest unit of the Manuherikia Group (Douglas, 1986). Douglas (1986) concluded that the St Bathans Member was deposited by braided rivers flowing in valleys incised into the basement rock (schist and greywacke). The St Bathans Member was not included in Mildenhall and Pocknall's (1989) palynological study of the Manuherikia Group, although one sample was documented in an unpublished report (Pocknall, 1982b). Pole and Douglas (1998) placed palynological samples from Grey Lake into their *Nothofagidites 'brassii' type* Zone and 'Myrtaceae-Arecaceae' Zone. The age, based on palynology and regional geology, is regarded as the earliest Miocene, ~19 ± 1 Ma and the successions contain diverse and well-preserved plant cuticles (e.g. Pole, 2007; 2008).
2.2. The Lauraceae leaf database

Our database includes 21 leaf specimens: three from Foulden Maar, nine from Mataura River and nine from Grey Lake (Fig. 2). The fossil specimens were placed in Lauraceae based on their leaf and cuticle morphology (Fig. 3A–D). The cuticle morphology is characterised by paracytic stomata with overarching subsidiary cells over the guard cells, with cuticular scales present in between (Hill, 1986). This cuticle morphology exists in the Australasian fossil record throughout the Cenozoic, as well as in the living Australasian Lauraceae (see e.g. Vadala and Greenwood, 2001; Pole, 2007).

The epidermal cuticle of the specimens in this study display a fairly generalized surface morphology (Fig. 3B–D). Epidermal cells are typically small, quadratic to rectangular-polygonal, normally ~10–20 μm × 8–12 μm, some slightly larger, generally with thick and straight cell walls (no undulation). Trichome attachment scars are common, sometimes abundant (Fig. 3D), mostly located on veins and occurring less abundantly on areoles. The paracytic stomata are easily identified and densely present in inter-vein areas. The stomatal pore length is ~7–8 μm and the guard cell length ~8–10 μm (Fig. 3C). The general shape of the stomatal complexes is rectangular-circular, often appearing considerably lighter in colour than surrounding epidermal cells, indicating thinner stomatal cuticle. Veins consist of rows of epidermal cells which are narrower and more elongated than areolar epidermal cells, typically ~7–8 × 20–25 μm. Trichome attachment scars are small, round, with thickened cuticle around the perimeter, surrounded by ~20 μm long epidermal cells in the anamocytic arrangement (Fig. 3B–D).
2.3. Laboratory methods

Sediment bulk samples were first disaggregated in hot water with added hydrogen peroxide (H₂O₂). The residues were subsequently sieved and fragmentary leaf material retained. The leaf fragments were then heated in concentrated hydrogen peroxide for several hours until cleared. Any adhering silica was removed with further hydrogen peroxide treatment of the leaf fragments and the cuticle stained with safranin. Finally, the fragments were mounted on microscope slides with thymol glycerine jelly. The mounted abaxial epidermal cuticle surfaces were photographed at ×200 magnification with a Leica camera (Leica DF310 FX), and associated software. Between five and seven images were taken of each specimen, evenly distributed across the mid-leaf areas, away from margins, midrib and veins, according to the recommended methodology of Poole and Kürschner (1999). These images form the database used for the results presented here. Lastly, each image was annotated using the software ImageJ (1.46h; http://imagej.nih.gov/ij) by engraving 300×300 µm or 200×200 µm squares on each image.

2.4. Stomatal analysis and calibration of palaeo-pCO₂

The stomatal proxy for palaeo-pCO₂ reconstruction is based on the observations of the inverse relationship that exists between frequency of stomata (pores on the leaf epidermis used for gas-exchange) and pCO₂, in order to minimize loss of water through transpiration (Woodward et al., 1987). This relationship is not universal, but is expressed in the large majority of woody plants (>90% in fossil studies; Royer, 2001). Stomatal frequencies can be quantified as stomatal density (SD: number of stomata per mm²) or stomatal index (SI: the proportion of stomata to all epidermal cells). It has been shown that in most cases SI more
reliably reflects \( p\text{CO}_2 \), whereas SD may be influenced by additional factors, e.g. light levels and water availability (Salisbury, 1927). The stomatal proxy has by now been applied to a wide variety of plant taxa from numerous different geological, palaeo-ecological and palaeo-climatological settings from the Palaeozoic until today and can be considered as a strong proxy for palaeo-\( p\text{CO}_2 \) (e.g. McElwain and Chaloner, 1996; Wagner et al., 1996; Beerling et al., 1998; McElwain, 1998; Royer et al., 2001; Wagner et al., 2002; Haworth et al., 2005; Roth-Nebelsick 2005; Kürschner et al., 2008; Barclay et al., 2010; Steinhorsdottir et al., 2011b; 2013; Grein et al., 2013; Bai et al., 2015; Mays et al., 2015; Steinhorsdottir and Vajda, 2015; Montañez et al., 2016; Steinhorsdottir et al. 2016a,b; Wolfe et al., 2016; McElwain and Steinhorsdottir, 2017). A strong argument for the reliability of SI tracking palaeo-\( p\text{CO}_2 \) is that cumulative mean statistical analyses show that typically less than seven counts of SI per fossil leaf are sufficient to establish a robust % value for that leaf, and – perhaps more importantly – a similarly small amount of leaves per time interval (i.e. sedimentary bed) yield a robust SI value for that time slice (Poole and Kürschner, 1999; Steinhorsdottir et al., 2011b). This robust relationship between SI and \( p\text{CO}_2 \) at a given time installs confidence in utilizing even small fossil leaf databases such as the one studied here.

Three methods of stomatal proxy-based palaeo-\( p\text{CO}_2 \) calibration are in use: 1) the empirical stomatal ratio method, which utilizes the ratio between the SI of fossil plants and the SI of extant nearest living relatives or equivalents (NLR or NLE), grown in known \( p\text{CO}_2 \) (natural or experimental), to estimate palaeo-\( p\text{CO}_2 \) (McElwain and Chaloner, 1995; McElwain et al., 1998); 2) the likewise empirical transfer function method, which relies on herbarium and/or experimental datasets of NLR/NLE responses to variations in \( p\text{CO}_2 \) to construct regression curves on which fossil SI can be plotted to infer palaeo-\( p\text{CO}_2 \) (Wagner et al., 1999; Royer, 2001; Beerling et al., 2002; Barclay and Wing, 2016); and 3) the mechanistic gas exchange modelling approach, which is taxon-independent and uses e.g.
In this study, we calibrated palaeo-$pCO_2$ using the stomatal ratio method and three separate transfer functions. Stomata and epidermal cells within annotated squares on the database images were counted (Fig. 3B) and SI calculated as $SI(\%) = \frac{SD}{(SD + ED)} \times 100$, where ED is the epidermal cell density. At least seven images, sensu Poole and Kürschner (1999) were used for mean SI determination for each specimen from each of the three stratigraphical levels, confirming the mean values by cumulative mean statistical analysis. Three previously published transfer functions (Kürschner et al., 2008) in addition to the stomatal ratio method (McElwain and Chaloner, 1995) were used. The transfer functions were constructed for Miocene extinct Lauraceae, based on extant nearest NLR or NLE species and in the case of the fossil species *Laurophyllum pseudoprinceps*, based on cross-calibration with the well-established NLE species *Laurus nobilis* and *Ginkgo biloba*, adding a correction factor where appropriate (see Kürschner et al., 2008 for details). The first transfer function is based on the extant species *Laurus nobilis* (laurel, leaves commonly referred to as ‘bay leaves’), independently calibrated from historical sets of herbarium material:

$$pCO_2_{\text{palaeo}} = 10^{3.173 - [0.5499 \times \log (SI_{\text{fossil}})]}$$

The second transfer function is also based on an extant species: *Ocotea foetens* (Tilo or ‘the stinkwood’), independently calibrated from herbarium material:

$$pCO_2_{\text{palaeo}} = 10^{2.9567 - [0.4284 \times \log (SI_{\text{fossil}})]}$$

The third transfer function is based on the extinct species *Laurophyllum pseudoprinceps*, established by cross-calibration with *L. nobilis* and *Ginkgo biloba*:

$$pCO_2_{\text{palaeo}} = -46.011 \times SI_{\text{fossil}} + 993.37$$

In addition, we use the stomatal ratio method of McElwain and Chaloner (1995), which utilizes the ratio between stomatal indices of fossil plants and their NLR or NLE in relation to
the ratio between known $p\text{CO}_2$ (modern) and palaeo-$p\text{CO}_2$. “Modern” $p\text{CO}_2$ was historically set at 300 ppm (defined as preindustrial $p\text{CO}_2$ concentration), but post-industrial $p\text{CO}_2$ can also be used when available with the appropriate SI. The ratio between Cretaceous and younger SI and modern SI is estimated to be 1:1 (McElwain and Chaloner, 1995) and the stomatal ratio calibration is thus expressed by the equation:

$$p\text{CO}_2\text{ palaeo} = \frac{\text{SI}_{\text{NLE}}}{\text{SI}_{\text{fossil}}} \times p\text{CO}_2\text{ modern.}$$

(4)

The here selected NLE is $L.\ nobilis$, with $\text{SI}_{\text{NLE}}$ of 18.32% at 300 ppm $p\text{CO}_2$ (Kürschner et al., 2008), resulting in:

$$p\text{CO}_2\text{ palaeo} = \frac{18.32}{\text{SI}_{\text{fossil}}} \times 300.$$  

(5)

Determining the genera of fossil Lauraceae is notoriously challenging, but even though cuticle evidence is proving useful (e.g. Ferguson, 1974; Christophel et al., 1996), the phylogenetic relationships of extant genera remains in some flux (e.g. van der Werff and Richter, 1996; Rohwer, 2000; Chanderbali et al., 2001). For this reason, although $L.\ nobilis$ (originally assigned as the specific NLE for extant species $Laurus\ abchasica$ in Kürschner et al., 2008) may not be the best NLE for the New Zealand Lauraceae studied here, we chose to utilize it as the NLE, for easier comparison of previously published results (Kürschner et al., 2008; Steinthorsdottir et al., 2016a, Steinthorsdottir et al., 2016b), effectively regarding it as a standardised Lauraceae.

3. Results

3.1. Stomatal indices and $p\text{CO}_2$ across the OMT

The fossil leaf cuticles from the oldest site, Foulden Maar from the OMT, have a mean SI of 7.5%, while the leaf cuticles from the two younger sites, Grey Lake and Mataura River, of
earliest and early Miocene age, show substantially higher SI at around 12 % (11.7 % and 12.2 % respectively) (Table 1). SI thus increased by >60% from the OMT into the early Miocene, implying considerably higher \( pCO_2 \) during the OMT than in the early Miocene.

\( \text{Table 1. here} \)

Atmospheric \( pCO_2 \) values obtained using the four separate calibrations are broadly inter-comparable within each of the time slices. Across the OMT, transfer function-derived \( pCO_2 \) estimates range from ~580 ppm using \textit{Ocotea foetens} to ~640–650 ppm using \textit{Laurus nobilis} and \textit{Laurophyllum pseudoprinceps} as NLE (Table 1; Fig. 4). Stomatal ratio-derived estimates using \textit{L. nobilis} show the highest calibrated OMT \( pCO_2 \) at ~730 ppm. Earliest and early Miocene \( pCO_2 \) records are by contrast considerably lower. Earliest Miocene transfer function-derived \( pCO_2 \) is lowest at ~430 ppm when calibrated using \textit{L. pseudoprinceps} as NLE, ~516–538 ppm when using \textit{L. nobilis} and \textit{O. foetens}, whereas stomatal ratio-derived \( pCO_2 \) estimates using \textit{L. nobilis} are intermediate at ~485 ppm (Table 1; Fig. 4). Early Miocene estimates indicate highly similar \( pCO_2 \) to that of the earliest Miocene, albeit slightly higher. Transfer function-derived \( pCO_2 \) is again lowest when calibrated using \textit{L. pseudoprinceps} at ~454 ppm, ranging ~520–542 ppm when using \textit{L. nobilis} and \textit{O. foetens}, and stomatal ratio-derived \( pCO_2 \) estimates using \textit{L. nobilis} are also again intermediate at ~490 ppm (Table 1; Fig. 4).

The overall average \( pCO_2 \) is thus ~650 ppm for the Oligocene–Miocene Transition and ~490–500 ppm for the earliest and early Miocene (~25% lower).

\( \text{Fig. 4 here} \)

Statistical analyses (two sample t-tests for equal means) of \( pCO_2 \) results reconstructed between each \( pCO_2 \) calibration and each time interval confirmed that earliest and early Miocene \( pCO_2 \) levels are very similar and thus cannot be separated statistically (p values 0.7–
0.9, null hypothesis confirmed), whereas the difference between the $pCO_2$ values derived for the OMT and those of the earliest and early Miocene $pCO_2$ values respectively are statistically significant ($p < 0.05$, null hypothesis rejected).

4. Discussion

4.1. Assessment of the new OMT $pCO_2$ record

An emerging concern with the increasing interest in stomatal proxy-based $pCO_2$ reconstructions is the question of how errors should be quantified. Traditionally errors have been reported as standard deviation or standard error, based on the spread of the data points from a mean. Although giving indication of the strength of the dataset presented, this clearly does not take into account potential inherent variability caused by e.g. taxa used (fossil vs modern species, and/or non species-specific records), evolutionary and environmental change, and possibly a range of other unknown factors. The community of researchers using the stomatal proxy for palaeo-$pCO_2$ reconstructions are working on this complex issue, but for now the best way forward may be to reconstruct $pCO_2$ using multiple taxa with a variety of available stomatal proxy methods, to reach the best consensus possible. It should be noted here that the small sample size of just three leaves from the Foulden Maar OMT locality results in smaller errors than for the larger samples from the Mataura River and Grey Lake localities (standard deviations, see Table 1) – contrary to the obvious assumption that smaller datasets are less likely to fully capture the natural error range – illustrates this issue well.

The fact however that $pCO_2$ values presented here, reconstructed using four calibrations with three NLE species in two distinct stomatal proxy methods, show a high degree of similarity within each sample (time slice), inspires confidence that Lauraceae SI
were responding to $pCO_2$ and that this response can be successfully utilized to reconstruct approximate levels of palaeo-$pCO_2$. The stomatal ratio method assumes a 1:1 response of stomata of Cenozoic and younger plants and their NLEs, therefore always recording changing $pCO_2$ levels when SI change (McElwain and Chaloner, 1996). Transfer functions however directly record plant SI responses to known $pCO_2$, including any non-linear responses, potentially providing more accurate calibrations (Royer, 2001; Beerling et al., 2002; Kürschner et al., 2008; Barclay and Wing, 2016). It has previously been shown that the stomatal ratio method and transfer functions produce similar results when used with the same leaf fossils (e.g. Steinthorsdottir et al., 2016b; McElwain and Steinthorsdottir, 2017). Here, we were unable to reconstruct $pCO_2$ using the gas exchange modelling approach (the third available stomatal proxy method), principally due to lack of $\delta^{13}C$ data for the leaf database. However, previous $pCO_2$ reconstructions using both the stomatal ratio method and the gas exchange model of Franks et al. (2014) with fossil leaf material from the late Pennsylvanian, recorded comparable $pCO_2$ values (Montañez et al., 2016), and the overall compatibility between $pCO_2$ results obtained using all the various methods (discussed below in section 4.2), suggests that the stomatal proxy methods generally produce comparable results. Standard deviations, measuring the dispersion of the data, are largest for the $pCO_2$ values calibrated using *Laurophyllum pseudoprinceps* as NLE in a transfer function, reflecting perhaps the non-linear response of this species (Table 1). *Laurus nobilis* was applied as NLE both in the stomatal ratio method and in the transfer function, recording higher variability when using the stomatal ratio method (highest $pCO_2$ at the OMT, but intermediate in the earliest and early Miocene when compared to the transfer function results). This may either indicate that the stomatal ratio method, which directly translates the SI signal to $pCO_2$, overestimates plant response to $pCO_2$, or that the transfer function used underestimates the response, and this needs to be further tested. The overall agreement
regarding the volume and direction of change of $pCO_2$ from the OMT and into the early Miocene does however indicate that the average $pCO_2$ reported here may be reflecting values approaching “true” palaeo-$pCO_2$.

4.2. Comparison with previously published OMT $pCO_2$ records

The few previously published stomatal proxy $pCO_2$ records largely agree with each other and with marine records regarding the trend and magnitude of $pCO_2$ across the OMT (see Fig. 1), with a few exceptions recording stable levels across the OMT. Considering that the stomatal $pCO_2$ records have been constructed using four calibrations with two stomatal proxy methods and various fossil plant species, this inspires confidence in the emerging pattern. Kürschner et al. (2008), using transfer functions with the stomatal densities of multiple herbarium and fossil plant species from Central Europe, including several Lauraceae, recorded a significant overall decrease in $pCO_2$ from ~600 ppm in the Late Oligocene to ~340 ppm in the early Miocene. Grein et al. (2013), using a leaf gas-exchange model for several fossil plant species from Germany, later reported two possible scenarios depending on which taxa were included in the calibrations: either an overall $pCO_2$ decrease from ~700 ppm in the latest Oligocene to ~500 ppm in the early Miocene when including Lauraceae in the fossil dataset, or considerably lower, stable $pCO_2$ of ~400 ppm across the OMT when excluding Lauraceae. Roth-Nebelsick et al. (2014), using a single species dataset with a leaf gas-exchange model from the late to latest Oligocene of Germany likewise recorded stable $pCO_2$ of ~400 ppm throughout the period. SI values do change during the studied intervals of Grein et al. (2013) and Roth-Nebelsick et al. (2014), suggesting that the $pCO_2$ signal may have been lost in the calibration. The model they use, the gas-exchange model of Konrad et al. (2008), has recently been tested with modern material and was shown to produce the most accurate
\(p\text{CO}_2\) estimates when used with multiple species, to derive a consensus \(p\text{CO}_2\) (Grein et al., 2013). Therefore, single-species records are perhaps not appropriate for this model, and the results of Grein et al. (2013) when using multiple fossil species, including Lauraceae, should be regarded as the more reliable. Steinthorsdottir et al. (2016a) used a single species dataset from the middle Eocene to latest Oligocene in the stomatal ratio method, and record \(p\text{CO}_2\) of ~475 ppm for the latest Oligocene.

Most recently, a high-resolution \(p\text{CO}_2\) record from Foulden Maar in New Zealand indicated a brief “spike” or doubling in \(p\text{CO}_2\) to ~1100 ppm at the OMT for ~20 kyr, during the termination of Mi-1 (Fig. 1) (Reichgelt et al., 2016) based on data derived using a separate leaf gas-exchange model (Franks et al., 2014), and (Reichgelt et al., 2016). This transient spike in \(p\text{CO}_2\) is interpreted to have occurred coincident with hydrological changes, i.e. increased moisture availability in the area (Reichgelt et al., 2016), a concurrence that has also been shown to have taken place during a doubling of \(p\text{CO}_2\) across the Tr–J boundary, due to the repression of stomatal transpiration (Steinthorsdottir et al., 2012). Reichgelt et al., 2016 also tested the responsiveness of their chosen NLE (\textit{Litsea calicara}) by plotting SD and SI derived from herbarium material to measured \(p\text{CO}_2\), but found that it was not responsive and therefore did not cross-check their \(p\text{CO}_2\)-record using additional stomatal proxy methods (stomatal ratio or transfer). We tested their published SI data with \textit{L. nobilis} as NLE in the stomatal ratio method, consistent with the methods used in this paper, and obtained very similar results to those presented by Reichgelt et al., (2016): a brief spike of ~1100 ppm, bounded by lower values of ~600 ppm before, and ~500 ppm after the spike. We do not record as high \(p\text{CO}_2\) as Reichgelt et al. (2016) in the present study, but speculate that this may be due to the lower stratigraphic resolution of our record, i.e. we record the increased \(p\text{CO}_2\) but not the full increase – i.e. the spike. Although the high \(p\text{CO}_2\) values recorded here are also based on leaves from the Foulden Maar section, potentially raising worries regarding specific
local conditions causing the remarkably low SD/SI and thus high calibrated \( pCO_2 \), the surrounding lower \( pCO_2 \) values from Foulden Maar in Reichgelt et al. (2016) and from the additional regional sites reported here mutually support the emerging pattern. However, the transient spike in \( pCO_2 \) at the OMT needs to be replicated using stomatal densities of fossil leaves from additional stratigraphic sections before full confidence can be declared.

4.3. Implications for climate dynamics at the OMT

The large negative excursion of oxygen isotopes at the termination of Mi-1 (Zachos et al., 2001b; Lear et al., 2004; Pälike et al., 2006; Liebrand et al., 2011; Mawbey and Lear, 2013), indicating rising temperatures and ice sheet retreat, has long been somewhat of a mystery, given the lack of a corresponding large \( pCO_2 \) excursion in the marine records (Zhang et al., 2013; Liebrand et al., 2017). Modelling studies meanwhile indicated that to force an ice-sheet retreat of the magnitude observed, greenhouse gas perturbations would be needed in addition to plate tectonics and orbital parameters (DeConto and Pollard, 2003; DeConto et al., 2008; Gasson et al., 2014, 2016), for example through carbon cycle feedback mechanisms (Mawbey and Lear, 2013). Clearly the existence of a transient doubling of \( pCO_2 \) as indicated by Reichgelt et al. (2016) and supported by the results presented here, would be of major interest in understanding the climate dynamics at play during the OMT and the termination of Mi-1. Understanding the sensitivity of Earth’s climate to \( pCO_2 \) is an extremely important research question with immense societal importance. The climate dynamics across the OMT are highly pertinent in this respect, as they happened in background \( pCO_2 \) similar to today’s, bringing the need for multiple high-fidelity records across climate transitions into sharp focus.
5. Conclusions

The Oligocene–Miocene transition (OMT) was a global cooling event characterized by expansion of Antarctic ice volume as well as transient ice sheet retreat episodes and associated climate warming. In New Zealand, the transition from the Oligocene to the Miocene is marked by a change from a low-diversity Nothofagus-dominated pollen assemblage to one of higher diversity, interpreted to reflect relatively cool climate followed by warmer conditions. Oscillating episodes of climate change across the OMT, including the transient Mi-1 glaciation, have long been difficult to reconcile with existing pCO₂ records, but recently, pCO₂ data for this interval suggested that the termination of Mi-1 was associated with a brief pulse of highly elevated pCO₂ (Reichgelt et al., 2016). The new pCO₂ record from the OMT into the early Miocene of New Zealand presented here confirms highly elevated pCO₂ across the OMT at ~650 ppm compared to the pCO₂ levels of ~500 ppm estimated for the earliest and early Miocene. This implies that pCO₂ may have played a more important role in the termination of the glaciation than previously thought. Although it is too early to assert whether a significant transient excursion in pCO₂ was, together with orbital parameters, the forcer in the termination of Mi-1, it is timely to state that this may have been the case, and should be investigated further.

Acknowledgments

We gratefully acknowledge funding from the Swedish Research Council (VR Starting Grant NT-7 2016 04905 to M.S and VR 2015 4264 to V.V) and from the Bolin Centre for Climate Research, Stockholm University to M.S. This research was further funded by Lund University Carbon Cycle Centre (LUCCI). M. Huber (Purdue University) is thanked for helpful discussions and C.H. Lear (Cardiff University) provided constructive feedback on an earlier version of the manuscript.
References


Larsson, L., Vajda, V., Dybkjaer, K., 2010. Vegetation and climate in the latest Oligocene-

Miocene climate evolution of northern Europe: A palynological investigation from

ice sheet dynamics and the global carbon cycle. Paleoceanography 19, PA4015.

Antarctic ice sheet and oceanographic response to eccentricity forcing during the early

Liebrand, D., de Bakker, A.T.M., Beddow, H.M., Wilson, P.A., Bohaty, S.M., Ruessink, G.,
Pälike, H., Batenburg, S.J., Hilgen, F.J., Hodell, D.A., Huck, C.E., Kroon, D., Raffi, I.,

Lindqvist, J.K., Lee, D.E., 2009. High-frequency paleoclimate signals from Foulden Maar,
Waipiata Volcanic Field, southern New Zealand: an Early Miocene varved lacustrine
diatomite deposit. Sediment. Geol. 222, 98–110.


Pocknall, D.T., 1982b. Pollen and spores from Blue Lake, St Bathans (H41) and Harliwicks Lignite Pit, Roxburgh (G43), Central Otago, New Zealand. Unpublished Report. Palynology Section, NZGS, Lower Hutt.


**Figure captions**

**Figure 1.** Overview of climate and $p$CO$_2$ reconstructions for the Oligocene and early–middle Miocene. The Mi-1 major cooling event is indicated. Redrawn after Zhang et al. (2013), who compiled published $p$CO$_2$ data, with stomatal proxy records principally from Beerling and Royer (2011). Benthic foraminifera $\delta^{18}$O isotope data from Zachos et al. (2008). Dashed lines indicate modelled $p$CO$_2$ glaciation thresholds for Antarctica (~750 ppm) and the Arctic/Northern Hemisphere (~280 ppm). New results showing transient elevated $p$CO$_2$ at the termination of Mi-1 from Reichgelt et al. (2016) indicated with a green dot at ~1100 ppm.

**Figure 2.** Location of the three fossil plant localities on the South Island of New Zealand.

**Figure 3.** Fossil Lauraceae leaf database. A: well-preserved leaf from the Grey Lake locality, belonging to genus *Cryptocarya*. Scale bar is 1 cm. B: epidermal surface of Lauraceae leaf specimen nr. SL 6272. The image shows the typical morphology of epidermal cells and stomata in the database. The turquoise rectangle illustrates how stomata (marked by dots) and epidermal cells were counted within engraved grids, to determine stomatal indices. Scale bar is 100 µm. C: close-up of an areola and a cluster of typical Lauraceae paracytic stomata. To the left of the areola, elongated vein epidermal cells are shown. Specimen nr. D 2322. Scale bar is 20 µm. D: epidermal surface showing abundant trichome scars. Specimen nr. SL 6276. Scale bar is 100 µm.
Figure 4. Reconstructed atmospheric $p$CO$_2$ across the Oligocene–Miocene transition and during the earliest, and early Miocene of New Zealand, using four separate calibrations (tree transfer functions and the stomatal ratio method) with three nearest living equivalent species. Earliest and early Miocene $p$CO$_2$ are highly comparable (average $p$CO$_2$ ~500 ppm), whereas $p$CO$_2$ for the latest Oligocene, near OMT, is significantly higher (average $p$CO$_2$ ~650 ppm). The data points are shown without error bars for visual clarity; see Table 1 for standard deviations.

Table 1. Stomatal indices and atmospheric $p$CO$_2$ across the OMT and into the early Miocene of New Zealand.
Table 1. Stomatal indices and atmospheric $p$CO$_2$ across the OMT and into the early Miocene of New Zealand.

<table>
<thead>
<tr>
<th>Locality</th>
<th>International zone</th>
<th>New Zealand zone</th>
<th>Age (Ma)</th>
<th>SI (%) Laura-ceae</th>
<th>$p$CO$_2$ (ppm): Transfer functions*</th>
<th>$p$CO$_2$ (ppm): Stomatal ratio**</th>
<th>Average $p$CO$_2$ (ppm)***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grey Lake</td>
<td>Early Miocene</td>
<td>Otaian</td>
<td>19 $^{\pm}2$</td>
<td>11.72 $^{\pm2.7}$</td>
<td>L. nobilis 542.3 $^{\pm9}$</td>
<td>454.2 $^{\pm22}$</td>
<td>501.8 $^{\pm8}$</td>
</tr>
<tr>
<td>Mataura River</td>
<td>Earliest Miocene</td>
<td>Late Watakian</td>
<td>22.5 $^{\pm1}$</td>
<td>12.24 $^{\pm3.1}$</td>
<td>O. foetens 519.8 $^{\pm11}$</td>
<td>490.8 $^{\pm10}$</td>
<td>492.3 $^{\pm18}$</td>
</tr>
<tr>
<td>Foulden Maar</td>
<td>Oligocene-Miocene transition</td>
<td>Watakian</td>
<td>23.2 $^{\pm0.2}$</td>
<td>7.53 $^{\pm5.5}$</td>
<td>L. pseudo-princeps 516.5 $^{\pm43}$</td>
<td>485.1 $^{\pm19}$</td>
<td>650.4 $^{\pm8}$</td>
</tr>
</tbody>
</table>

*, **Four $p$CO$_2$ calibrations were used: three transfer functions and the stomatal ratio method. The results are recorded with standard deviations, with the exception of age estimates, which are based on previous geological studies. ***The results indicate that $p$CO$_2$ levels were considerably higher at the OMT, close to the termination of the Mi-1 glaciation, compared to the earliest and early Miocene levels.
Highlights

- A New Zealand fossil *Lauraceae* leaf database is from the OMT and early Miocene
- OMT $pCO_2$ was reconstructed using the stomatal proxy method
- $pCO_2$ was ~650 ppm at the OMT and decreased to ~500 ppm into the early Miocene
- Geological evidence show transient glaciation episode Mi-1 termination at OMT
- Results indicate $pCO_2$ played a larger role than previously assumed in termination
$p$CO$_2$ perturbation across the New Zealand Oligocene–Miocene transition

Graphics Abstract
Figure 4

Legend

Transfer functions
- Laurus nobilis
- Ocetea foetens
- Laurophyllum pseudoprinceps

Stomatal ratio
- Laurus nobilis
- Average $pCO_2$ (all)