Fossil plant stomata indicate decreasing atmospheric CO$_2$
prior to the Eocene-Oligocene boundary

M. Steinthorsdottir$^1$, A.S. Porter$^2$, A. Holohan$^2$, L. Kunzmann$^3$, M. Collinson$^4$, and J.C. McElwain$^2$

$^1$Department of Geological Sciences and Bolin Centre for Climate Research, Stockholm University, 106 91 Stockholm, Sweden
$^2$School of Biology and Environmental Science, Earth Institute, University College Dublin, Dublin 4, Ireland
$^3$Museum of Mineralogy and Geology, Senckenberg Natural History Collections Dresden, Germany
$^4$Department of Earth Sciences, Royal Holloway University of London, Egham, Surrey, United Kingdom

Abstract

A unique stratigraphic sequence of fossil leaves of *Eotrigonobalanus furcinervis* (extinct trees of the beech family, Fagaceae) from central Germany has been used to derive an atmospheric pCO$_2$ record with multiple data points spanning the late middle to late Eocene, two sampling levels which may be earliest Oligocene, and two samples from later in the Oligocene. Using the inverse relationship between the density of stomata and pCO$_2$, we show that pCO$_2$ decreased continuously from the late middle to late Eocene, reaching a relatively stable low value before the end of the Eocene. Based on the subsequent records, pCO$_2$ in parts of the Oligocene was similar to latest Eocene values. These results suggest that a decrease in pCO$_2$ preceded the large shift in marine oxygen isotope records that characterizes the Eocene-Oligocene transition and that when a certain threshold of pCO$_2$ change was crossed, the cumulative effects of this and other factors resulted in rapid temperature decline, ice build up on Antarctica and hence a change of climate mode.

1 Introduction

1.1 The role of pCO$_2$ in Cenozoic climate
The Cenozoic era is characterized by large climatic variations, including the fundamentally important transition from an ice-free ‘greenhouse’ planet to the modern ‘icehouse’ planet with polar glaciations. This climatic transition is generally thought to have been driven primarily by changes in pCO$_2$ and/or the thermal isolation of Antarctica by the opening of Southern Ocean gateways (DeConto and Pollard, 2003; Zachos et al., 2008; Hansen et al., 2013; Hren et al., 2013; Goldner et al., 2014; Inglis et al., 2015). However, the full extent of the role of pCO$_2$ in Cenozoic climate change remains unresolved. The most detailed Cenozoic temperature and pCO$_2$ records are derived from marine isotope proxies (e.g. Foster et al., 2012; Pagani et al., 2011; Pearson et al., 2009; Zachos et al., 2001, 2008). Isotope records, however, may be influenced by a variety of taphonomic and diagenetic biases (see Coxall and Pearson, 2007 for review; and Pagani et al., 2011), that can obscure the climatic signal, and thus need independent evaluation by separate proxy records (Berling and Royer, 2011).

Eocene temperatures were globally much higher than today, leading to a weakened equator-to-pole temperature gradient and muted seasonal cycle compared to today; the so-called ‘Eocene equable climate problem’ (Sloan and Barron, 1992; Huber and Caballero, 2011). Climate modelling has been able to reconstruct this climatic pattern only with excessively high pCO$_2$ levels (~4500 ppm: Huber and Caballero, 2011), but such elevated pCO$_2$ atmospheres do not agree with most proxy records. It has therefore been speculated that Eocene climate sensitivity was elevated compared to today and/or that other forcing in addition to high pCO$_2$ was involved (Caballero and Huber, 2013; Hansen et al., 2013). In order to solve this enigma reliable multiple proxy records of pCO$_2$ are of paramount importance.

The fundamental climatic reorganization that occurred close to the Eocene-Oligocene boundary (33.8 Ma), often referred to as the Eocene-Oligocene transition (EOT, 34-33.5 Ma), had drastic consequences for biological systems. These included both terrestrial and marine faunal and floral extinctions accompanied by evolutionary turnover (Prothero, 1994; Coxall and Pearson, 2007; Sheldon et al., 2009; Kunzmann, 2012; Kvaček et al., 2014), although vegetation changes in the European terrestrial record appear to be less dramatic and more gradual (Kvaček et al., 2014; Kunzmann et al., submitted). General circulation models of Paleogene climate have shown that continuously declining pCO$_2$, amplified by Milankovitch forcing and ice-albedo feedbacks, could cause significant temperature reduction. This could result in a permanent continental Antarctic ice-sheet once a critical pCO$_2$ threshold, generally
considered to be < 700 ppm is crossed e.g. (DeConto and Pollard, 2003; Coxall et al., 2005; Pollard and DeConto, 2005; Zachos and Kump, 2005; Pagani et al., 2011; Hansen et al., 2013). Modeling studies thus indicate that lowering of pCO\textsubscript{2} may have been the primary forcer of this cooling transition (DeConto and Pollard, 2003; DeConto et al., 2008). However, detailed estimates for pCO\textsubscript{2} for the Eocene and the Oligocene are highly variable and sometimes contradictory or showing unexpected relationships with paleo-temperature proxy records (see Pagani et al., (2005)). For example, comparing the pCO\textsubscript{2} record of Pearson et al., (2009: Fig. 1), which is based on measurements of Boron isotopes in planktonic foraminifera, and the benthic foraminifera oxygen isotope (d\textsuperscript{18}O) compilations of Zachos et al., (2008), it is evident that in the late Eocene d\textsuperscript{18}O-inferred deep ocean cooling coincided with decreasing pCO\textsubscript{2}. In contrast, there is little evidence of warming in the early Oligocene, despite a surprising initial large increase in pCO\textsubscript{2}. Overall, the pCO\textsubscript{2} and O isotope-based temperature records seem to be (largely) coupled in the Eocene, but decoupled in the Oligocene. Pagani et al. on the other hand recently published compiled alkenone-based pCO\textsubscript{2} records and found declining pCO\textsubscript{2} before and during the Antarctic glaciation (EOT and earliest Oligocene) (Pagani et al., 2011: Fig. 4), supporting the role of pCO\textsubscript{2} as the primary forcing agent of Antarctic glaciation, consistent with model derived thresholds. A compounding factor of these discrepancies is that the influence of temperature on ice sheet volume is unconstrained and the influence of temperature versus ice volume the d\textsuperscript{18}O record is unresolved, with no proxy identified to isolate ice sheet volume changes, complicating further the interpretation of the climate proxy datasets. Independent proxy records of E-O pCO\textsubscript{2} are therefore desirable and may support one or the other of the major prevailing scenarios outlined above, or provide alternative information on Cenozoic climate change.

One of the four proxies that have been identified as being particularly useful for Cenozoic pCO\textsubscript{2} reconstructions by the Intergovernmental Panel on Climate Change (initially reported in the 4\textsuperscript{th} IPCC report, 2007) is the terrestrial proxy based on stomatal densities of fossil plants. Previous studies using the stomatal proxy method of pCO\textsubscript{2} reconstructions for the part of the Cenozoic relevant here were, however, mostly of low resolution and have been inconclusive. Some suggested that pCO\textsubscript{2} was essentially stable at between 300 and 450 parts per million by volume (ppm) during the Eocene, Oligocene and Miocene (Royer, 2001; Royer et al., 2001; Greenwood et al., 2003; Maxbauer et al., 2014) and others suggesting distinct decrease in pCO\textsubscript{2} across the Eocene-Oligocene boundary (Retallack, 2001). More recent studies suggest higher and possibly rapidly decreasing pCO\textsubscript{2} (ranging ca. 1000-500 ppm) during the late
middle Eocene (Doria et al., 2011; Grein et al., 2011). In this issue, Liu et al. report a “late Eocene” pCO$_2$ from a single stratigraphical level of ca. 390 ppm. However, the chronological range they supply for their pCO$_2$ estimate (42.0-38.5 Ma) falls within the late Lutetian to Bartonian in the Middle Eocene, thus recording an unusually low pCO$_2$ estimate for this time-interval characterized by high temperatures (Liu et al., 2015). Closer to the E-O boundary, one study suggests that pCO$_2$ was significantly higher at the EOT than during the early Oligocene (Roth-Nebelsick et al., 2004) and others that early Oligocene to early Miocene pCO$_2$ was ca. 400 ppm throughout (Grein et al., 2013; Roth-Nebelsick et al., 2014).

Here we present a new stomatal proxy-based record with multiple data points spanning the late middle to late Eocene, two sampling levels that according to current available evidence are from the earliest Oligocene, and two samples from later in the Oligocene.

1.2 The stomatal proxy method of paleo-pCO$_2$ reconstruction

Stomata are pores on plant leaf surfaces through which gas exchange takes place; i.e. carbon is obtained from CO$_2$ and at the same time water vapour and oxygen are lost by diffusion. An inverse relationship exists between the frequency of stomata and pCO$_2$, as established by Woodward (1987) from observations of herbarium material, showing that modern tree species have responded to the anthropogenic rise in pCO$_2$ by reducing their stomatal frequency significantly. The inverse relationship between stomatal frequency, recorded as ‘stomatal density’ (SD = the number of stomata per mm$^2$) or ‘stomatal index’ (SI = the percentage of stomata relative to epidermal cells), and pCO$_2$ has been repeatedly demonstrated for a wide variety of plant taxa from disparate geological and ecological settings from the Palaeozoic until today and is thus established as a strong proxy for paleo-pCO$_2$ (e.g. Beerling et al., 1998; McElwain, 1998; Retallack, 2001; Royer et al., 2001; Kürschner et al., 2008; Steinthorsdottir et al., 2011b; 2013; Steinthorsdottir and Vajda, 2015). The increasingly close match between stomatal proxy pCO$_2$ results and independent proxy records, actual pCO$_2$ measurements and in some cases climate modelling (e.g. Finsinger and Wagner-Cremer, 2009; Foster et al., 2012; Kürschner et al., 2008; Retallack, 2001; Rundgren and Björck, 2003; Steinthorsdottir and Vajda, 2015) instils growing confidence in stomatal frequency for recording past pCO$_2$. Strongly supporting the validity of the stomatal proxy is also the identification of the mechanism by which plants control their stomatal densities based on atmospheric pCO$_2$. All plants use the enzyme carbonic anhydrase to detect pCO$_2$ around their leaves (Frommer,
mature leaves (early shoots) then control stomatal development of younger leaves through long-distance signalling (Lake et al., 2002), involving the HIC gene signalling pathway (Brownlee, 2001; Gray et al., 2000).

In order to transform stomatal frequency data derived from fossil plants into paleo-pCO$_2$ estimates it is usually necessary to compare stomatal data from present day plants that are either phylogenetically related or in other ways equivalent to the fossil plants. Nearest living relatives (NLR) should be used when possible, but when these cannot be identified for the fossil plants, nearest living equivalents (NLE= present day species that are of comparable ecological setting and/or structural similarity to their fossil counterpart) may be used instead (McElwain and Chaloner, 1995; Barclay et al., 2010; Steinthorsdottir et al., 2011a; Steinthorsdottir et al., 2011b).

There are three stomatal paleo-pCO$_2$ calibration methods in use. These are (i) the ‘stomatal ratio method’ (McElwain and Chaloner, 1995; McElwain, 1998), which relies on a ratio between stomatal frequencies of fossil plants and their NLE to semi-quantify pCO$_2$; (ii) the ‘transfer function method’, which relies on herbarium material and/or experimental datasets for NLR/NLE responses to calculate pCO$_2$ curves (e.g. Beerling and Royer, 2002); and (iii) the more recently developed taxon-independent ‘mechanistic gas exchange modelling’ approach (e.g. Wynn, 2003; Konrad et al., 2008; Franks et al., 2014; Grein et al., 2013; Roth-Nebelsick et al., 2014) which all use measurements of stomatal density and pore size to estimate maximum theoretical gas exchange rates, together with various photosynthetic biochemical traits, and in some cases palaeoenvironmental information, to estimate palaeo-CO$_2$. The stomatal ratio method, which is used here, calibrates paleo-pCO$_2$ based on two so-called standardizations. The first is the ‘modern’ standardization that assumes that the ratio between past and modern pCO$_2$ is 1 (R$_{CO_2} = 1$) and is applied to young material, typically from the Quaternary. The second is the ‘Carboniferous’ standardization that sets the ratio between past and modern pCO$_2$ at two times preindustrial levels of 300 ppm (R$_{CO_2} = 2 = 600$) (McElwain and Chaloner, 1995). Both standardizations are usually applied to fossil leaf material of Cenozoic age and older to yield minimum and maximum pCO$_2$ estimates and both standardizations will be used in this paper.

We have chosen not to apply the mechanistic optimization model of Konrad et al., (2008) to our study, because it has recently been shown in a modern test of the model to produce the most accurate pCO$_2$ estimates when used on multiple species, to derive a
consensus pCO$_2$ estimate from their area of overlapping pCO$_2$ values (Grein et al., 2013), and
we here study a one-species database. The optimization model produces very large and
species-dependent uncertainty in pCO$_2$ estimates when applied to individual fossil species
(Konrad, 2008; Roth-Nebelsick et al., 2012) and even modern species (Grein et al., 2013) for
which all the biochemical, environmental and anatomical parameters required to initialize the
model are known (Konrad, 2008; Grein et al. 2013; Roth-Nebelsick et al., 2012). We have
also not applied the mechanistic stomatal model of Franks et al. (2014) because it is shown to
be highly sensitive to initial parameterization of assimilation rate resulting in +/- 500 ppm
error in palaeo-pCO$_2$ estimates (McElwain et al., 2015b). Future work on *Eotrigonobalanaus furcinervis* will aim to constrain likely paleo-assimilation rate for this extinct taxon by
applying available paleo-assimilation proxies (McElwain et al. 2015a; 2015b; Wilson et al.,
2015) and undertaking elevated pCO$_2$ experiments on appropriately selected NLEs.

2 Material and methods

2.1 Fossil leaf database

*Eotrigonobalanus furcinervis* (Rossm. 1840) Walther et Kvaček in Kvaček and Walther 1989,
an extinct evergreen Fagaceae, (Fig. 1) existed from the middle Eocene to the Oligocene-
Miocene boundary and was geographically widely distributed, i.e. from central Europe to
Russia, as well as to the Mediterranean area (Mai and Walther, 2000; Velitzelos et al., 1999).
It is considered as a thermophilous species that grew in evergreen broadleaved forests as well
as in mixed mesophytic forests adapted to humid and warm-temperate to subtropical climate
(Mai and Walther, 2000). *E. furcinervis* was present in megafossil assemblages or
“taphocoenoses” derived from riparian forests, back swamps, peat bogs and zonal vegetation
and therefore the parent plant tolerated a wide range of water table conditions and soil
characteristics. Whereas in the Eocene it often predominated in zonal Fagaceae-Lauraceae
forests (Mai and Walther 2000), in the Oligocene mixed mesophytic forest it was ecologically
sub-dominant. Based on the combined fossil record of cupules, seeds and leaves, including
cuticles, it is commonly accepted that the fossils represent a single long-lived but rather
variable fossil species, although minor changes in leaf anatomy have led to the distinction of
two subspecies, ssp. *furcinervis* (mainly Eocene, rare in Oligocene) and ssp. *haselbachenses*
(only Oligocene; Kvaček and Walther 1989). The latter is distinguished by the absence of
pubescence (trichome clusters) on the abaxial leaf epidermis. Furthermore, a variety of leaf morphotypes can be distinguished that have been interpreted as ecological variants (ecotypes, see Kriegel, 2001).

Except for the material from the Kleinsaubernitz site (Fig. 2), the leaf specimens used here originate from the central German Weiβelster Basin (Fig. 2), a coastal alluvial plain at the southern margin of the North German–Polish ‘Tertiary’ Basin (Standke, 2008). This basin is well-known for its extensive record of middle Eocene to early Miocene plant assemblages that are mainly derived from azonal vegetation, i.e. riparian and swamp forests (e.g. Mai and Walther, 2000; Kunzmann, 2012). The Knaau assemblage represents the fluvial hinterland of the Weiβelster lignite swamps (Mai and Walther, 2000).

The leaves used here are derived from a succession of cuticle rich taphocoenoses that contain *E. furcinervis* ranging in age from the late middle Eocene to the end of the Oligocene (Table 1, Fig. 3). The database analysed here consists of 233 *E. furcinervis* leaf cuticle fragments on as many slides, representing 151 separate individual leaf specimens (Appendix A and Table 2). All specimens represent material used in previous taxonomic-systematic studies, they are housed in the Senckenberg Natural History Collections Dresden, Germany. The plant fossil assemblages have been positioned on the most recent lithostratigraphy for central and East Germany (Standke, 2008; Standke et al., 2010; Fig. 3, Table 2) using published information on the fossil sites (Mai and Walther, 1991; Mai and Walther, 2000; Kunzmann and Walther, 2002; Hennig and Kunzmann, 2013; Ferdani, 2014) and personal observations (LK). Information on dating is provided in section 2.2 below.

One late Oligocene locality, Kleinsaubernitz (Figs. 2, 3A), lies within the Lausitz basin, at its southern margin or even in the hinterland (Standke, 2008). Leaf specimens derive from a sediment-filled maar, volcanic in origin, preserving a parautochthonous assemblage mainly representing zonal vegetation (Walther, 1999) in contrast to the mainly azonal vegetation from the coastal plains of the Weiβelster Basin.

### 2.2 Stratigraphy and dating

The relative stratigraphic positions for the samples from the Weiβelster Basin (Figs. 2, 3A) are based on accumulating knowledge from more than 150 years of geological-paleontological investigations of the respective units (see Walther and Kunzmann, 2008 for summary). The samples are derived from a superposed sequence of four lignite seams and their associated strata (Table 1, Fig. 3A) the subdivisions of which can be readily recognized across different opencast mines.
It is not possible to directly correlate the plant fossil bearing horizons in the Weißenster Basin to the global marine stratigraphy. Although there are a number of brackish-marine intercalations (Standke et al., 2010) most of these strata lack fossils suitable for biostratigraphy. As is typical for lignite-bearing non-consolidated sedimentary successions (i.e. gravel, sands, silts, clays) hard parts of mineralised organisms that might be used for biostratigraphy in continental sequences (such as mammals and charophytes) are lacking due to dissolution by humic acids originating from organic material. Non-consolidated sediments do not reveal any casts or molds of these former fossils. This is also the case for any intercalation of brackish-marine sediments in the Weißenster Basin profile. The lack of common index fossils prevents accurate stratigraphic chronology in the basin and reduces the level of stratigraphic resolution compared with that typically attainable for marine deposits (e.g. Roth-Nebelsick et al., 2014). Furthermore, heterogeneity in facies types (channel, floodplain, tidal deposits, swamps) and in grain sizes of the sediments precludes the use of magnetostratigraphic methods which need longer sequences of fine-grained sediments without facies shifts (e.g. lake sediments) to produce reliable data.

Based on a series of consecutive pollen assemblages in the Weißenster Basin strata a regional phytostratigraphic concept was developed (Krutzsch, 1967) that can be applied to all Formations, Members and submembers, and also to all lignite seams and even individual seam measures (Krutzsch, 2011). All of our investigated material is unambiguously assigned to a certain unit of the regional lithostratigraphic scheme (Fig. 3A) and thus connected to a respective pollen zone or subzone (Fig. 3A, Table 1). However, the pollen zonation yields only a relative age for a given horizon within the regional palynostratigraphic framework and does not enable correlation to global stratigraphy or to the global time scale. The attempt by Krutzsch (2011) to correlate the Eocene spore-pollen zones with the global timescale is used herein (Fig. 3A) as it is the only available information to interpret our assemblages. A ‘late’ Eocene age (i.e. late Bartonian + Priabonian, Krutzsch 2011) for our respective assemblages has been previously inferred based on floristic comparison to assemblages from the nearby Bohemian basins (Czech Republic) some of which have absolute dates from volcanic rocks (i.e. Kučín, Staré Sedlo, Roudníky; Kvaček et al. 2014).

In the younger part of the succession, marine deposits have yielded index fossils suitable for biostratigraphy. Marine strata above the Gröbers Member of the Böhlen Formation are placed into regional dinoflagellate zones D13 and D14 (Köthe, 2005; Standke et al. 2010) which are Rupelian in age. The Haselbach horizon of the Gröbers Member, including our assemblage sites Schleenhain 4 and Haselbach 2 (figs 2 and 3A), was therefore
interpreted to be basalmost Oligocene (Standke et al. 2010; Krutzsch 2011), however, the only definitive information from the dinoflagellate data is that the samples must be older than mid Rupelian. Lithofacies changes in the centre of the Weiβelster Basin, i.e. the profile in the Schleenhain mine (Kunzmann and Walther, 2002) that indicate major sea level changes below the sample horizon of sites Schleenhain 4 and Haselbach 2 are consistent with those that occur around the Eocene - Oligocene boundary and are documented in other European successions (e.g. Hooker et al., 2009). A basalmost Oligocene age for the Schleenhain 4 and Haselbach 2 sites is also indicated by the first occurrence of *Boehlensipollis hohlii* in the sampled horizon which places the sample in spore-pollen zone 20A/B sensu Krutzsch (2011).

*Boehlensipollis hohlii* is regarded as a key element for the Oligocene in central and East Germany (Krutzsch, 2011) and had also been treated as such in the International Geological Correlation Programme (Vinken, 1988). However, it should be mentioned that Collinson (1992) reported several occurrences of the species in the late Eocene of the UK and Frederiksen (1980) reported the species ranging from late middle Eocene to Oligocene in the USA. Possibly the species arose in the USA and spread later via the UK into central Europe but further work is needed to securely link the occurrences of *Boehlensipollis hohlii* with the marine biostratigraphy and the global time scale. In short, there are two independent pieces of evidence (lithofacies, first appearance of *Boehlensipollis hohlii*) that clearly suggest an early Oligocene age for the Schleenhain 4 and Haselbach 2 samples. However, this is not conclusive evidence and direct linkage to the global marine scale is currently not available. The site at Kleinsaubernitz has been located on Figure 3 based on its pollen assemblage which is zone 20G (Goth et al., 2003).

In summary, the material from the Weiβelster Basin comes from a superposed sequence where relative stratigraphic position is securely known (Table 1). Relative changes of SD (and thus pCO₂) through the succession can be placed in context of the spore-pollen zonation. However, the positions of the Eocene-Oligocene boundary and the Oligocene-Miocene boundary cannot be located with certainty in the Weiβelster profiles. All age estimates in figures 3 and 4 are based on Krutzsch’s (2011) proposed correlation of the regional spore-pollen zones to global sea level changes. Independent support is needed for these proposals so they should be regarded as preliminary age information.

### 2.3 Stomatal density quantification
Cuticles were prepared at the Senckenberg Natural History Collections Dresden as a part of an existing collection. Cuticle slides were prepared using standard methods for Palaeogene material. Fragments removed from leaf specimens with preparation needles were macerated for 1-4 minutes in Schulze’s solution. Cuticles were then neutralized with NH₄OH, washed with distilled water, and upper and lower cuticles were separated using preparation needles. Finally, the cuticles were stained with Safranin and affixed to slides by glycerol jelly. For this study, the slides were examined microscopically by an adaptation of the methodology set out by Poole and Kürschner (1999) in order to determine SD. According to this protocol, counts from mid lamina are preferable in establishing SD, but the fragmented nature of a proportion of the fossil material did not allow establishing where individual cuticle samples were located on the original leaf surface (see Fig. 1B). Individual epidermal cells were not easily discernible in the majority of the *E. furcinervis* material, making SI determination impossible.

SD was obtained using a Nikon SK Light Microscope at x200 magnification with a graticule providing a counting field of 0.042 mm². The graticule was centred over areas where stomata occurred in greatest numbers (away from veins and margins where those were known, *sensu* Poole and Kürschner, 1999) and up to five individual counts were recorded for each slide, resulting in 659 SD counts for the database of 151 leaf specimens (Table 1 and Appendix 1). Data was stored in Microsoft Excel 2010 before being statistically manipulated using MINITAB (version 16.1.1 for Windows).

### 2.4 Choice of Nearest Living Equivalent and Paleo-pCO₂ calibration

*Eotrigonobalanus furcinervis* belongs to the Fagaceae, but its phylogenetic position is not well defined. Based on cupule morphology, *Eotrigonobalanus* belongs to a basal clade of the family, exhibiting intermediate characters between modern *Trigonobalanus* and *Castanopsis* (Mai, 1995). However, leaf venation and leaf cuticle micromorphology place *Eotrigonobalanus* with *Trigonobalanus* and *Lithocarpus*, away from *Castanopsis* (Kvaček and Walther, 1989), an affiliation recently confirmed by Denk et al., (2012). Since the phylogeny of Fagaceae has changed considerably (Manos et al., 2001; Manos et al., 2008), an improved systematic framework is still required to confirm the phylogenetic position of *Eotrigonobalanus*. Because the exact relationship of *Eotrigonobalanus* to crown group Fagaceae is unknown, a single nearest living relative (NLR) could not be obtained, hence the
nearest living equivalent (NLE) approach has been used for the stomatal proxy-based pCO$_2$
reconstruction.

In this study, *Trigonobalanus doichangensis* was chosen as the NLE, due to it being a
basal species within the Fagaceae family and having leaf macro-morphological and leaf
cuticle micro-morphological similarities with *E. furcinervis*, including cyclocytic stomata and
similarly structured trichomes (Kvaček and Walther, 1989; see also Denk et al., 2012). Two
herbarium specimens of *T. diochangensis*, formerly collected in 1988, were sampled at the
Kew Herbarium (Royal Botanical Gardens, Kew, Richmond, Surrey, UK). Approximately
1 cm$^2$ was cut from mid-lamina of each leaf specimen and dry mounted onto a slide. Five
cuticle images from each slide were taken at 200x magnification using a Leica DM2500
epifluorescent microscope with Leica DFC300FX camera (Leica® 312 Microsystems,
Wetzlar, Germany) and Syncroscopy Automontage (Syncroscopy Ltd, Cambridge, UK)
digital imaging software. A 0.09 mm$^2$ square was superimposed on each image and stomatal
density was determined within this square following the protocol of Poole and Kürschner
(1999). SD was determined to be 546.11/mm$^2$ at pCO$_2$ of 351 ppm (collection year levels
according to NOAA ESRL data, available at www.esrl.noaa.gov).

Using the stomatal ratio method with *T. doichangensis* NLE for *E. furcinervis*, we
calibrated paleo-pCO$_2$ using the equations below to derive minimum and maximum paleo-
pCO$_2$ (‘Modern’ and ‘Carboniferous’ Standardization of McElwain and Chaloner, 1995),
respectively:

\[
\text{Paleo-pCO}_2\text{min} (\text{ppm}) = ((\text{SD}_{\text{NLE}}=546.11)/\text{SD}_{\text{fossil}}) \times 351 \text{ ppm} \\
\text{Paleo-pCO}_2\text{max} (\text{ppm}) = ((\text{SD}_{\text{NLE}}=546.11)/\text{SD}_{\text{fossil}}) \times 600 \text{ ppm}
\]

3 Results

3.1 Stomatal density and paleo-pCO$_2$ estimates

SD of *E. furcinervis* range between ca. 425 and 740 stomata/mm$^2$. The lowest SD values
(signifying highest pCO$_2$) are found in the oldest deposits, late middle to earliest late Eocene
(spore-pollen zone 17), and the highest values (signifying lowest pCO$_2$) are found in the later
late Eocene (spore-pollen zone 18o), representing the most pronounced SD change during the
time period covered by the dataset (Table 2, Fig. 3B), with three intermediate samples
showing intermediate values (spore-pollen zones 17/18, 18u, 18uo). During this interval SD increases by >300 stomata/mm$^2$ or by ca. 75%, a very significant change indicating a sizeable decrease in pCO$_2$ in perhaps ca. 3.5 million years. Stomatal densities then decrease slightly again and remain around 600-650/mm$^2$ in the latest Eocene and in samples that may be earliest Oligocene as well as in the late Oligocene (spore-pollen zones 19, 20A/B, 20G, II). At the end of the Oligocene, SD decreases again to ca. 570/mm$^2$.

Paleo-pCO$_2$ calibrated using the stomatal densities of *E. furcinervis* will be discussed as average values and evaluated in terms of relative change, as introduced above. The largest change in palaeo-pCO$_2$ is the decrease from the late middle to earliest late Eocene of >250 ppm, from ca. 630 ppm to ca. 365 ppm – a decrease in pCO$_2$ of ca. 40% (Fig. 3B; Table 2). Concentrations of CO$_2$ then increase again by ca. 45 ppm to ca. 410 ppm in the latest Eocene and possibly earliest Oligocene, and further to between ca. 430-475 ppm in the late and latest Oligocene (Fig. 3B; Table 2).

4 Discussion

4.1 Fidelity of the Saxony stomatal pCO$_2$ record

The Saxony fossil leaf database is unique in that this relatively large database derives from a well-constrained stratigraphic succession and consists of a single species throughout – *E. furcinervis* – which is the most ideal situation when using fossil leaf material to reconstruct paleo-pCO$_2$, since inter-species variability is eliminated and stomatal responses to pCO$_2$ are likely to be consistent through time. The procurement of a single-species dataset from multiple stratigraphic levels across several million years is not common, in particular when the stratigraphy represents time intervals of significant climate and/or environmental change, as is the case here. The principal challenge concerning the Saxony stomatal density record was translating the stomatal signal into reliable levels of pCO$_2$. One of the main limitations associated with the use of paleo-proxies is the preservational state of fossil material and in this case the preservation of fossil leaves did not allow paleo-pCO$_2$ reconstruction using gas exchange models for independent comparison of the results using the stomatal ratio method because stomatal pore length could not be measured in all samples with confidence. Additionally, there is a lack of available transfer functions for potential NLEs of *E. furcinervis*, so it was not possible either to obtain independent pCO$_2$ reconstructions using the
The stomatal ratio method has however been shown to closely match results produced with transfer function methods (Beerling and Royer, 2002; Royer, 2003; Barclay et al., 2010; Steinthorsdottir et al., 2011b) and is seen as a good alternative where detailed estimates of other photosynthetic parameters, which are required to initialize mechanistic models, are not readily available (McElwain, unpubl.).

The absence of an obvious NLE for *E. furcinervis* – an extinct species of uncertain phylogenetic affinity – further introduces potential errors in pCO$_2$ calibration. Although we consider *T. doichangensis* the best available NLE, there is no guarantee that its stomatal density and degree of response to pCO$_2$ closely mirrors that of its distant fossil relative. The pCO$_2$ levels calibrated here appear somewhat low compared to most previously published pCO$_2$ datasets, although broadly comparable to stomatal pCO$_2$ records (Fig. 4A). When testing three additional potentially suitable NLE species for reconstructing pCO$_2$ using the Saxony database; *Trigonobalanus verticillata, Castanopsis cuspidata* and *Lithocarpus henryi*, the resulting paleo-pCO$_2$ values were extremely low – considerably lower than when using the chosen NLE *T. doichangensis* – in many cases being lower than minimum pCO$_2$ levels required to maintain sufficient plant growth and reproduction (i.e. below the ecological compensation point). This indicates that, for some reason (e.g. species-specific responses) the stomatal proxy-derived pCO$_2$ estimates presented here based on *E. furcinervis* may be artificially low.

### 4.2 Comparison with vegetation and proxy continental climate records

Paleoclimate reconstructions based on Central European megafloras reveal a sharp decline in continental cold month mean temperature (Mosbrugger et al., 2005) and mean annual temperature (Moraweck et al., 2015; Kvaček et al., 2014) in the late Eocene (Fig. 4B) which is consistent with the timing of the pCO$_2$ decline that we report here (Figs. 4A,B), and with global sea surface temperature trends as recorded by marine oxygen-isotopes (Fig. 4C). The marine isotope curve also shows a gradual decrease of temperatures in the late Eocene, but in contrast with the terrestrial records, the most pronounced and abrupt change coincides with the Eocene-Oligocene boundary (Fig. 4C), suggesting that pCO$_2$ drawdown may have taken place gradually before the slow feedback ice sheet growth was initiated and global temperatures dropped suddenly in response. The possibility remains that future terrestrial
proxy reconstructions of pCO$_2$ will record a transient major drawdown of pCO$_2$ at the Eocene-Oligocene boundary. In order to resolve this, more proxy records from well-constrained Early Oligocene sites must be added.

Furthermore, palaeo-vegetation analysis of the Weiβelster and North Bohemian basins reveals that gradual restructuring of dominantly evergreen forests by immigration of deciduous species such as *Platanus neptuni*, *Trigonobalanopsis rhamnoides* and *Taxodium dubium* (Kunzmann et al. submitted) took place in the late Bartonian to early Priabonian interval around ca. 38 Ma (Kvaček, 2010; Teodoridis and Kvaček, in press). The temporal coincidence of pCO$_2$ decline and major vegetation transition – from angiosperm-dominated notophyllous evergreen forests to mixed mesophytic forests – suggests a potential causal role of pCO$_2$ decline in the changing ecological composition of forests. It may have been in part triggered by differential responses of evergreen and deciduous taxa to declining pCO$_2$ (Fig. 4A,B), explaining the lag between “temperatures” indicated by terrestrial vegetation and sea surface temperatures recorded by marine oxygen-isotopes (Fig. 4C). The functional trait of deciduousness is an adaptation to episodic cooling (Zanne et al., 2014). However, it has also been demonstrated experimentally (McElwain et al., 2015) and on theoretical grounds (Niinemets et al., 2011) that taxa with low leaf mass per area or LMA (i.e. those that are deciduous or herbaceous) and high stomatal conductance have faster photosynthetic rates than evergreens at lower atmospheric pCO$_2$. In contrast, evergreens have higher responsiveness in terms of photosynthetic rates at elevated pCO$_2$ (Niinemets et al., 2011). A transition from elevated to lower CO$_2$ atmospheres would therefore favour the ecophysiology of deciduous or low LMA taxa over evergreen high LMA species. Further experimental investigation is now required to tease apart the relative importance of ‘CO$_2$ starvation’ and increased temperature seasonality on the late Bartonian to early Priabonian vegetation transition.

4.3 Comparison with other pCO$_2$ records

Previously published stomatal proxy-based pCO$_2$ records from the part of the Cenozoic relevant to this paper do not always agree, but instead report highly elevated (McElwain, 1998; Doria et al., 2011; Grein et al., 2011; Smith et al., 2010), intermediate (Retallack, 2009) or similar to modern (Royer et al., 2001) pCO$_2$ for the Eocene. Similarly high variability in estimated pCO$_2$ levels exists for the Oligocene as well as the Miocene (Grein et al., 2013; Kürschner et al., 2008; Roth-Nebelsick et al., 2014; Royer et al., 2001). The results reported
here are the highest stratigraphic resolution pCO$_2$ estimates derived from the late Eocene to early Miocene basins in Saxony (see Table 2, Fig. 1 and 3). Previous studies have tended to only report temporal trends on stomatal parameters (Roth-Nebelsick et al., 2004) or to lump pCO$_2$ estimates from single Saxony localities into coarse temporal bins making cross comparison difficult (Roth-Nebelsick et al., 2012). However, where individual site pCO$_2$ data are reported (Grein et al., 2013) our estimates are in very good agreement with previous studies despite differences in species and calibration approach (Table 2). For example, Grein et al. (2013) report pCO$_2$ estimates of ~400 ppm and between ~430 to ~530 ppm respectively for the sites Kleinsaubernitz and Witznitz (Fig. 3) using the Konrad et al. (2008) stomatal optimization model in a consensus approach on multiple species (3–4) including E. furcinervis (Table 2). The optimization model produces a very large range of pCO$_2$ estimates however (~270 to 710 ppm) when applied to E. furcinervis alone from stratigraphically lumped samples from Haselbach and Profen (Table 2) (Roth-Nebelsick et al., 2012). In comparison with the study of Roth-Nebelsick et al. (2012), we report seven stratigraphically well-resolved pCO$_2$ estimates spanning the same interval for which they report a single lumped average (~470 ppm) for 2 sites (Table 2). This is thus the first study therefore to resolve a significant drop in paleo-pCO$_2$ in the late Eocene, prior to the E-O boundary from a stratigraphically well constrained and relatively high-resolution record.

Using a rigorous generalized statistical framework, Beerling et al. (2009) revised previously published pCO$_2$ estimates based on Ginkgo and Metasequoia from the early Eocene and middle Miocene upwards by 150-250 ppm. Based on this revision, average stomatal proxy-based pCO$_2$ is 450-700 ppm in the Paleogene and 500-600 ppm in the Neogene (Beerling et al., 2009). Interestingly, the younger set of pCO$_2$ estimates was fully compatible to marine proxy data and modelling results (e.g. Pagani et al., 2005; Hansen et al., 2008), whereas the older set of estimates seemed to underestimate pCO$_2$ compared to the other approaches, even after the upwards revision of stomatal pCO$_2$ values (see Fig. 4 in Beerling et al., 2009). However, Kürschner et al. (2008) indicated that an upwards correction of 150-200 ppm – a so-called ‘correction factor’ – was necessary also when reconstructing Miocene paleo-pCO$_2$ with two species from the Lauraceae family. Recently discrepancies between the various pCO$_2$ proxies have narrowed significantly, and a coherent pattern of long-term Cenozoic pCO$_2$ has emerged, indicating pCO$_2$ mostly in the hundreds rather than thousands of ppm, although shorter-term inter-proxy discrepancies remain (see Beerling and
Royer, 2011, Fig. 1). It has thus become evident that pCO$_2$ values reconstructed using the stomatal proxy do not require a correction factor.

Pearson et al. (2009) reconstructed pCO$_2$ for the late Eocene to early Oligocene using the planktonic foraminifera boron isotope pH proxy and found that the main reduction in pCO$_2$ took place before the main phase of EOT ice growth (ca. 33.6 Ma: DeConto et al., 2008), followed by a sharp recovery to pre-transition levels and then a more gradual decline. Their results thus support the central role of declining pCO$_2$ in Antarctic ice sheet initiation and development and agree broadly with carbon cycle modelling (e.g. Merico et al., 2008). The quantitative estimates of pCO$_2$ varied greatly however, according to which d$^{11}$B value was used to derive pH, with geochemical models of the boron cycle suggesting a range of 37-39‰ for sea water (sw) d$^{11}$B during this time (Simon et al., 2006). The range of pCO$_2$ values spanned from ca. 2000-1500 ppm at the upper end and ca. 620-450 ppm at the lower end (Pearson et al., 2009). Recently published alkenone-based pCO$_2$ records found significantly declining pCO$_2$ before, as well as during, the Antarctic glaciation (EOT and earliest Oligocene), supporting the pCO$_2$ pattern of Pearson et al. (2009) and the role of pCO$_2$ as the primary forcing agent of Antarctic glaciation, consistent with model derived thresholds (DeConto et al., 2008; Pagani et al. 2011; Zhang et al., 2013). The alkenone-derived dataset values are overall higher – but not much higher – than those derived by stomatal densities, with late Eocene values of ca. 1000 ppm, minimum value of ca. 670 at 33.57 Ma and then gradual decline to ca. 350 ppm at the Oligocene-Miocene boundary.

In general therefore, Cenozoic stomatal proxy-based pCO$_2$ values, reconstructed using the available methods, tend to report somewhat lower pCO$_2$ values than alkenone- or boron-based proxies as well as those from mass balance modelling. As discussed above, isotope-based proxy records depend on a range of assumptions that influence the output interpretation to a large extent. In addition, it has recently been shown that the modelled pCO$_2$ threshold for Antarctic glaciation at the EOT, routinely referred to be ca. 700 ppm (DeConto and Pollard, 2003), is in fact highly dependent on the type of climate model used and the configurations of the model (Gasson et al., 2014), implying that the range of Cenozoic pCO$_2$ may be due for an update. It is noteworthy that most existing stomatal proxy-based pCO$_2$ records report a similar range of low pCO$_2$ values for this time interval and an internally consistent pattern is emerging for the Cenozoic (see Fig. 4A). Stomatal proxy-based pCO$_2$ records that are independently calibrated using different species/genera and families usually agree with one
another and show Eocene-Miocene pCO\textsubscript{2} in the range of 800-300 ppm (Fig. 4A). Although this discrepancy between proxies needs to be better understood before significant reevaluation of the role of pCO\textsubscript{2} in Cenozoic climate change is warranted, it should not be a priori rejected that collectively stomatal proxy records may accurately indicate lower pCO\textsubscript{2} levels during the Cenozoic than previously assumed.

### 4.4 Implications for Cenozoic climate sensitivity

The concept of Earth’s climate sensitivity – usually defined as the equilibrium surface temperature response to doubling of pCO\textsubscript{2} (2xCO\textsubscript{2}) – is a key parameter for understanding the mechanisms of future climate change. Recently there has been much focus on accurately and uniformly defining this concept, but although progress has been made, discrepancies still remain. The term most in use for predicting future climate change is “equilibrium climate sensitivity”, defined as the response of global mean surface temperatures to a 2xCO\textsubscript{2} radiative forcing after all the fast feedbacks have occurred (changes in atmospheric temperatures, clouds, water vapor, winds, snow, sea ice, etc.), but before the slow feedbacks occur (mainly ice sheet, vegetation and the carbon cycle responses) and often estimated to be ca. 3°C (Rohling et al., 2012; Royer et al., 2012; Hansen et al., 2013; Huber et al., 2014; the Intergovernmental Panel on Climate Change report 2013 best estimate 1.5–4.5 °C). When studying paleo-climate sensitivity, which has the potential to be accurately inferred from high-resolution paleo-climate proxy archives, both fast and slow feedbacks must be considered to define a related concept – the “Earth System sensitivity”, where e.g. pCO\textsubscript{2} may act both as forcer and as feedback, and which depends to a large degree on the initial climate state (Royer et al., 2007; Hansen et al., 2013). In the Cenozoic, pCO\textsubscript{2} is involved in climate change both as forcing and feedback, with evidence of increased climate sensitivity in warm climates, rather than cool ones (Hansen et al., 2013).

The Eocene-Oligocene global cooling transition is represented by a large increase in deep-sea benthic foraminiferal oxygen isotope values, reflecting simultaneously decrease in temperatures and increased ice sheet growth, with as of yet no proxy to accurately separate the relative effects of the two (Zachos et al., 2001; 2008). Constraining the decrease in temperature that occurred during the transition is thus a work in progress, but consensus is emerging around a ca. 2–5 °C cooling in sea surface as well as mean annual air temperature (e.g. Lear et al., 2008; Zachos et al., 2008; Liu et al., 2009; Bohaty et al., 2012; Wade et al.,
The EOT cooling and glaciation was forced by a decrease in pCO$_2$ from ca. 1000 ppm to ca. 600 ppm based on marine isotopes and climate modelling (e.g. DeConto et al., 2008; Pearson et al., 2009; Pagani et al., 2011) or ca. 800 ppm to ca. 400 ppm based on stomatal records (e.g. Beerling and Royer, 2011; this dataset) – a decrease of at least ca. 40% in pCO$_2$ in < 5 Ma. A simple estimation of Earth System sensitivity during the EOT suggests elevated sensitivity compared to today, implying an enhancing factor by fast and/or slow feedbacks, such as ice sheet growth, but the radiative contribution of each is presently unknown (Lunt et al., 2010; Goldner et al., 2012; Gasson et al., 2014; Maxbauer et al., 2014). The transition in Earth’s climate mode from the Eocene greenhouse to the Oligocene icehouse was driven by changes in pCO$_2$ (and associated feedbacks) that largely fall within the range of modern to predicted future pCO$_2$ – albeit in opposite direction. Understanding how the Earth System responds to radiative forcing within this range (i.e. understanding Earth System sensitivity) is of considerable interest, with the input and correlation of multiple paleo-pCO$_2$ proxy records being of crucial importance.

5 Conclusions

The new terrestrial stomatal proxy-based pCO$_2$ record presented here, derived from fossil leaves of *Eotrigonobalanus furcinervis* (extinct Fagaceae, beech tree family) from Saxony, Germany, spans the late middle Eocene to latest Eocene, with two sampling levels which are probably from earliest Oligocene, and two samples from later in the Oligocene. The record indicates that pCO$_2$ decreased continuously and gradually by ca. 40% during the late Eocene, from ca. 630 ppm in the late middle Eocene to ca. 365 ppm in the late Eocene and ca. 410 ppm near the Eocene-Oligocene boundary. Late and latest Oligocene pCO$_2$ was slightly higher at around 430-475 ppm. Although the pCO$_2$ values reported here may be artificially low, due to factors inherent to stomatal proxy-based calibration, they nonetheless broadly agree with the pCO$_2$ range of previously published Eocene-Miocene stomatal proxy records, indicating that Cenozoic pCO$_2$ may have been considerably lower than previously thought based on marine proxies. The substantial late Eocene decrease in pCO$_2$ reported here is consistent with terrestrial records of vegetation change and reconstructions of coldest month mean temperatures, as well as with marine isotope records of global sea surface temperatures. The substantial drop in temperatures and/or ice sheet growth that defines the Eocene-
Oligocene boundary in the marine record is not recorded here. This may be caused by the possibility that the Saxony record does not possess the stratigraphic resolution to record such a change, or indicate that decrease in pCO$_2$ took place before the recorded decrease in global sea surface temperatures. The results reported here lend strong support to the theory that pCO$_2$ drawdown, rather than continental reorganization, was the main forcer of the Eocene-Oligocene climate change, when a ‘tipping point’ was reached in the latest Eocene, triggering the plunge of the Earth System into icehouse conditions.

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Table 1. Lithostratigraphic and phytosstratigraphic positions of the *Eotrigonobalanus furcinervis*-containing fossil taphocoenoses in the Weiβelster Basin (central Germany); lithostratigraphy after Standke et al. 2010, spore-pollen zonation after Krutzsch (2011).

<table>
<thead>
<tr>
<th>Assemblage / site</th>
<th>Reference for fossil flora</th>
<th>Formation</th>
<th>Member</th>
<th>Horizon</th>
<th>Epoch</th>
<th>Spore-pollen zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Witznitz</td>
<td>Mai and Walther 1991</td>
<td>Cottbus</td>
<td>Thierbach</td>
<td>Witznitz</td>
<td>end of Oligocene</td>
<td>II</td>
</tr>
<tr>
<td>Espenhain-Störmthal</td>
<td>Mai and Walther 1991</td>
<td>Cottbus</td>
<td>Thierbach</td>
<td>Witznitz</td>
<td>end of Oligocene</td>
<td>II</td>
</tr>
<tr>
<td>Haselbach 2</td>
<td>Mai and Walther 1978</td>
<td>Böhlen</td>
<td>Gröbers</td>
<td>Haselbach</td>
<td>earliest Oligocene?</td>
<td>20A/B</td>
</tr>
<tr>
<td>Schleenhain 4</td>
<td>Kunzmann and Walther 2012</td>
<td>Böhlen</td>
<td>Gröbers</td>
<td>Haselbach</td>
<td>earliest Oligocene?</td>
<td>20A/B</td>
</tr>
<tr>
<td>Schleenhain 3</td>
<td>Kunzmann and Walther 2002</td>
<td>Borna</td>
<td>Domsen</td>
<td>overlying bed of lignite seam 23o</td>
<td>latest Eocene</td>
<td>19 (?)</td>
</tr>
<tr>
<td>Schleenhain 2</td>
<td>Ferdani 2014, Mai and Walther 2000</td>
<td>Borna</td>
<td>Bruckdorf</td>
<td>underlying bed of lignite seam 23o and leaf measure in lignite seam 23o</td>
<td>late Eocene</td>
<td>18o</td>
</tr>
<tr>
<td>Haselbach 1</td>
<td>Mai and Walther 2000</td>
<td>Borna</td>
<td>Bruckdorf</td>
<td>intercalated bed between lignite seam 23u and 23o</td>
<td>late Eocene</td>
<td>18uo</td>
</tr>
<tr>
<td>Schleenhain 1</td>
<td>Hennig and Kunzmann 2013</td>
<td>Borna</td>
<td>Bruckdorf</td>
<td>overlying bed of lignite seam 23u</td>
<td>late Eocene</td>
<td>18u</td>
</tr>
<tr>
<td>Knau</td>
<td>Mai and Walther 2000</td>
<td>Borna</td>
<td>uncertain</td>
<td>fluvial deposit</td>
<td>late Eocene</td>
<td>17/18</td>
</tr>
<tr>
<td>Profen-Süd</td>
<td>Fischer in Mai and Walther 2000</td>
<td>Profen</td>
<td>Wallendorf</td>
<td>underlying bed of lignite seam 1</td>
<td>late middle Eocene</td>
<td>17</td>
</tr>
</tbody>
</table>
Table 2. The Saxony *Eotrigonobalanus furcinervis* database, including spore-pollen zones (Krutzsch 2011) and epoch inferred from them, stomatal density counts and pCO$_2$ calibration results, all shown with standard deviation, average pCO$_2$ in bold. Comparison to previously published stomatal proxy-based pCO$_2$ results from central Germany and nearby regions listed in the far right column.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Epoch</th>
<th>Spore/pollen zone</th>
<th>SD (stomata/mm$^2$)</th>
<th>pCO$_2$ Min (ppm)</th>
<th>pCO$_2$ Max (ppm)</th>
<th>pCO$_2$ Average (ppm)</th>
<th>No. of leaves</th>
<th>Other studies CO$_2$ ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Witznitz, Espenhaien-Störmthal</td>
<td>latest Oligocene</td>
<td>II</td>
<td>569.02 ± 108.40</td>
<td>351.6 ± 79.12</td>
<td>600.02 ± 135.03</td>
<td>475.81 ± 107.08</td>
<td>45</td>
<td>−420 to −530 ppm$^1$</td>
</tr>
<tr>
<td>Kleinsaubernitz</td>
<td>late Oligocene</td>
<td>20G</td>
<td>623.29 ± 97.82</td>
<td>316.8 ± 58.41</td>
<td>540.71 ± 99.7</td>
<td>428.76 ± 79.05</td>
<td>25</td>
<td>−400 ppm$^1$</td>
</tr>
<tr>
<td>Schleenhain 4, Haselbach 2</td>
<td>earliest Oligocene (?)</td>
<td>20 A/B</td>
<td>657.13 ± 118.98</td>
<td>302.5 ± 59.31</td>
<td>516.29 ± 101.23</td>
<td>409.40 ± 80.27</td>
<td>21</td>
<td>n/a</td>
</tr>
<tr>
<td>Schleenhain 3</td>
<td>latest Eocene</td>
<td>19</td>
<td>642.88 ± 84.05</td>
<td>303.1 ± 35.54</td>
<td>517.24 ± 60.66</td>
<td>410.17 ± 48.10</td>
<td>11</td>
<td>n/a</td>
</tr>
<tr>
<td>Schleenhain 2</td>
<td>late Eocene</td>
<td>18 α</td>
<td>740.65 ± 148.90</td>
<td>269.56 ± 53.01</td>
<td>460.05 ± 90.48</td>
<td>364.80 ± 71.74</td>
<td>39</td>
<td>n/a</td>
</tr>
<tr>
<td>Haselbach 1</td>
<td>late Eocene</td>
<td>18 uo</td>
<td>505.88 ± 47.06</td>
<td>373.50 ± 35.99</td>
<td>637.45 ± 61.43</td>
<td>505.48 ± 48.72</td>
<td>2</td>
<td>−470 (ave.$^2$)</td>
</tr>
<tr>
<td>Schleenhain 1</td>
<td>late Eocene</td>
<td>18 u</td>
<td>661.18 ± 90.93</td>
<td>296.15 ± 44.65</td>
<td>505.43 ± 76.20</td>
<td>400.79 ± 60.42</td>
<td>4</td>
<td>n/a</td>
</tr>
<tr>
<td>Knau</td>
<td>late Eocene</td>
<td>17/18</td>
<td>495.50 ± 77.80</td>
<td>397.33 ± 68.7</td>
<td>678.12 ± 117.25</td>
<td>537.73 ± 92.98</td>
<td>4</td>
<td>n/a</td>
</tr>
<tr>
<td>Profen-Süd</td>
<td>late middle Eocene</td>
<td>17</td>
<td>426.14 ± 83.56</td>
<td>467.87 ± 101.78</td>
<td>798.51 ± 173.71</td>
<td>633.19 ± 137.74</td>
<td>1</td>
<td>−710 (max)$^2$</td>
</tr>
</tbody>
</table>

$^1$Applying Konrad et al. (2008) stomatal optimization model in a multispecies consensus approach (Grein et al., 2013)

$^2$Applying Konrad et al. (2008) stomatal optimization model to stratigraphically lumped *Eotrigonobalanus furcinervis* samples from Profen and Haselbach (Roth-Nebelsick et al., 2012). n/a = Individual site CO$_2$ data not reported so direct comparison not possible.
Figures

Figure 1. *Eotrigonobalanus furcinervis* (Rossm. 1840) Walther et Kvaček in Kvaček and Walther 1989, A: mass occurrence of leaves in lignite, Schleenhain opencast mine, Saxony, Germany, site Schleenhain 2, Borna Formation, Bruckdorf Member, late Eocene (Priabonian), SPP zone 18o, MMG PB SchleOE 535; B: abaxial leaf cuticle with stomata and trichome bases, Schleenhain opencast mine, Saxony, Germany, site Schleenhain 4, Böhlen Formation, Gröbers Member, earliest Oligocene (Rupelian), SPP zone 20A/B, slide MMG PB SchleMO 11/05 from leaf SchleMO 556/2.
Figure 2: Sites (asterisks) of *Eotrigonobalanus furcinervis*-containing fossil taphocoenoses in central and east Germany considered in the present investigations, note: the Schleenhain and Haselbach opencast mines revealed taphocoenoses in four and two distinct lithostratigraphic positions respectively (see also stratigraphic chart in fig. 3). Map legend: D = Germany, CZ = Czech Republic, PL = Poland, FR = France, NL = the Netherlands.

Figure 3. Stratigraphic position of the assemblages with *Eotrigonobalanus furcinervis*, regional lithostratigraphy and Krutzsch’s (2011) correlation to the spore-pollen zones including his proposed correlation of spore-pollen zones to global scale (see text section 2.2 for explanation and comments on dating uncertainty); black vertical bars next to assemblage names are the temporal uncertainty (based on a combination of lithostratigraphic information of the respective unit and spore-pollen zonation); bars of Schleenhain 1 and 2 are not to scale because gaps in the sediment deposition of the respective units are not equivalent to the duration of spore-pollen zones; gaps between the Eocene spore-pollen (sub-)zones illustrate gaps in the terrestrial sediment record, i.e. erosion. For horizon information see table 1.
Figure 4. CO₂, vegetation and climate trends through the Cenozoic. The most significant changes in pCO₂, forest ecosystem composition (A) and continental climate as tracked by terrestrial plants (B) take place in the late Eocene, whereas the most significant change in global temperatures as tracked by marine isotopes (C) takes place at the Eocene-Oligocene boundary, indicating that the significant climate transition at the Eocene-Oligocene boundary was preceded by a gradual decrease in pCO₂ during the late Eocene. (A): pCO₂ estimates from fossil stomata (this study pink with black error bars) in the context of existing stomatal proxy estimates (in grey from Beerling and Royer, 2011) in a chronostratigraphic framework. Vertical bar shows the gradual late Eocene vegetational restructuring of the dominantly evergreen forests of the Weißelster and North Bohemian basins studied here (dark green to light green), suggesting a potential causal role of pCO₂ decline in the changing ecological forest composition (*Kunzmann and Walther, 2012; **Kvaček et al., 2014; Kunzmann et al., submitted). Note that the assigned ages for CO₂ values from this study are estimated based on the biostratigraphic controls presented in Fig 3. Absolute ages were not available for any of the nine fossil study sites (Table 2) although clear superposition information is available throughout allowing good estimates of the temporal sequence of CO₂ estimates (see Fig 3). (B): Continental temperature curve: Record of continental cold month mean temperature for Central Europe during the last 45 My, redrawn from Mosbrugger et al (2005). Horizontal bars
represent coexistence intervals. Orange curve shows data from the Weißenster and Lausitz Basins, northeast Germany; blue curve shows data from the Lower Rhine Basin, northwest Germany (see Mosbrugger et al., 2005 for details). (C): Global climate (temperature) curve derived from stacked records of deep-sea benthic foraminiferal oxygen-isotopes: a proxy for relative changes in marine temperature in the late Eocene prior to ice build up, based on updated records from Deep Sea Drilling Project and Ocean Drilling Program sites. Raw data is smoothed by using 15-point running mean, to minimize biases introduced by uneven temporal and spatial distribution of records (data from Zachos et al., 2001; 2008; and references therein).