Palaeogeography, Palaeoclimatology, Palaeoecology 441 (2016) 653–658

Contents lists available at ScienceDirect

Palaeogeography, Palaeoclimatology, Palaeoecology

journal homepage: www.elsevier.com/locate/palaeo

Was atmospheric CO_2 capped at 1000 ppm over the past 300 million years?

J.C. McElwain [a,*], I. Montañez [b], J.D. White [c], J.P. Wilson [d], C. Yiotis [a]

[a] Earth Institute, O' Brien Centre for Science, School of Biology and Environmental Science, University College Dublin, Ireland
[b] Department of Earth and Planetary Sciences, University of California, Davis, USA
[c] Department of Biology, Baylor University, Waco, TX, USA
[d] Department of Biology, Haverford College, Haverford, USA

ARTICLE INFO

Article history:
Received 23 April 2015
Received in revised form 14 October 2015
Accepted 15 October 2015
Available online 23 October 2015

Keywords:
Carbon dioxide
Stomata
Plant assimilation
Photosynthesis
CO_2 proxy
Carbon isotopes

ABSTRACT

Atmospheric carbon dioxide concentration has shifted dynamically over the Phanerozoic according to mass balance models and the majority of proxy estimates. A new paleo-CO_2 proxy method underpinned by mechanistic understanding of plant stomatal, isotopic and photosynthetic responses to CO_2 has provocatively claimed that maximum paleoatmospheric CO_2 was capped at 1000 ppm for the majority of the past 300 million years. Here we evaluate the robustness of the new paleo-proxy CO_2 model by testing its sensitivity to initial parameterization and to scaling factors employed to estimate paleophysiological function from anatomical and morphological traits. A series of sensitivity analyses find that the model is robust to modification in some of the constants employed, such as CO_2 compensation point and mesophyll conductance, resulting in variability in paleo-CO_2 estimates which are already accounted for in the error propagation of the model. We demonstrate high sensitivity in the model to key input parameters such as initial fossil plant assimilation rate, termed A_0 and scaling factors used to estimate stomatal conductance from measurements of fossil stomata. Incorrect parameterization of A_0 has resulted in under estimation of pCO_2 by as much as 600 ppm. Despite these uncertainties, our analysis highlights that the new mechanistic paleo-CO_2 proxy of Franks et al. (2014) has significant potential to derive robust and more accurate CO_2 estimates from fossil plant stomata, as long as parameterization of A_0 is strongly justified with species appropriate morphological and anatomical data. We highlight methods that can be used to improve current estimates of fossil plant assimilation rates, reduce uncertainty associated with implementation of the Franks et al. (2014) model and importantly add to understanding of patterns of plant productivity over the Phanerozoic, for which there currently is no consensus.

1. Introduction

Atmospheric composition has fluctuated markedly throughout Earth history driven by changes in major sources and sinks of carbon, sulfur, phosphorous and oxygen (Bergman et al., 2004; Berner, 2006; Montañez and Soreghan, 2006). Beyond ice core measurements, there are no direct methods available for estimating paleoatmospheric composition. 'Proxy' methods have been developed which provide indirect estimates of gaseous components of Earth's atmosphere as far back as one billion years ago (Ekart et al., 1999; Royer et al., 2004; Pagani et al., 2005; Barclay et al., 2010; Breecker et al., 2010; Steinthorsdottir et al., 2011; Schubert and Jahren, 2012). Atmospheric evolution has also been modeled using mass balance approaches and knowledge of biogeochemical cycles (Berner, 1990; Berner, 2006). Both proxy and model estimates have converged regarding the 'big picture' atmospheric fluctuations, however many critical uncertainties remain. Reconstructing the deep-time dynamics of atmospheric carbon dioxide (c_a) has received the greatest attention because of the obvious importance of this greenhouse gas in driving and amplifying global climate change. There is a pressing need to better constrain how sensitive global climate is to a doubling of CO_2 as this will largely determine the amplitude of global temperature increase over the next century as CO_2 continues to rise (Hansen et al., 2008).

A new paleo-CO_2 proxy method (Franks et al., 2014) uses the relationship between plant carbon assimilation rate (A_n) and c_a derived from gas exchange measurements (Eq. (1)) (Farquhar and Sharkey, 1982; Von Caemmerer, 2000) to infer paleo-CO_2 in the geological past from fossil plant stomatal anatomy and both leaf and atmospheric carbon isotopic composition.

$$c_a = \frac{A_n}{g_{c(tot)} \cdot (1 - c_i/c_a)} \quad (1)$$

* Corresponding author.
E-mail addresses: Jennifer.mcelwain@ucd.ie (J.C. McElwain), ipmontanez@ucdavis.edu (I. Montañez), Joseph_D_White@baylor.edu (J.D. White), jwilson@haverford.edu (J.P. Wilson), chyiotis@gmail.com (C. Yiotis).

http://dx.doi.org/10.1016/j.palaeo.2015.10.017

Table 1
Input parameters and scaling factors used to estimate paleo-CO_2 from fossil plants in the sensitivity analysis of Franks et al. (2014).

Species	Control [a]	Experiment 1 (S3)	Experiment 2 (S4)	Experiment 3(A_0)
Aglaophyton major	$A_0 = 3$ μmol m^{-2} s^{-1} S3 = 0.6, S4 = 0.2	$A_0 = 3$ μmol m^{-2} s^{-1} S3 = 0.4, S4 = 0.2	$A_0 = 3$ μmol m^{-2} s^{-1} S3 = 0.6, S4 = 0.4	$A_0 = 6$ μmol m^{-2} s^{-1} S3 = 0.6, S4 = 0.2
Asteroxylon Mackie	$A_0 = 3$ μmol m^{-2} s^{-1} S3 = 0.6, S4 = 0.2	$A_0 = 3$ μmol m^{-2} s^{-1} S3 = 0.4, S4 = 0.2	$A_0 = 3$ μmol m^{-2} s^{-1} S3 = 0.6, S4 = 0.4	$A_0 = 6$ μmol m^{-2} s^{-1} S3 = 0.6, S4 = 0.2
Macroneuropteris scheuchzeri	$A_0 = 6$ μmol m^{-2} s^{-1} S3 = 0.6, S4 = 0.2	$A_0 = 6$ μmol m^{-2} s^{-1} S3 = 0.4, S4 = 0.2	$A_0 = 6$ μmol m^{-2} s^{-1} S3 = 0.6, S4 = 0.4	$A_0 = 13$ μmol m^{-2} s^{-1} S3 = 0.6, S4 = 0.2
Neuropteris ovata	$A_0 = 6$ μmol m^{-2} s^{-1} S3 = 0.6, S4 = 0.2	$A_0 = 6$ μmol m^{-2} s^{-1} S3 = 0.4, S4 = 0.2	$A_0 = 6$ μmol m^{-2} s^{-1} S3 = 0.6, S4 = 0.4	$A_0 = 16$ μmol m^{-2} s^{-1} S3 = 0.6, S4 = 0.2
Dicroidium elongatum	$A_0 = 6$ μmol m^{-2} s^{-1} S3 = 0.6, S4 = 0.2	$A_0 = 6$ μmol m^{-2} s^{-1} S3 = 0.4, S4 = 0.2	$A_0 = 6$ μmol m^{-2} s^{-1} S3 = 0.6, S4 = 0.4	$A_0 = 10$ μmol m^{-2} s^{-1} S3 = 0.6, S4 = 0.2
D. odontopteroides	$A_0 = 6$ μmol m^{-2} s^{-1} S3 = 0.6, S4 = 0.2	$A_0 = 6$ μmol m^{-2} s^{-1} S3 = 0.4, S4 = 0.2	$A_0 = 6$ μmol m^{-2} s^{-1} S3 = 0.6, S4 = 0.4	$A_0 = 10$ μmol m^{-2} s^{-1} S3 = 0.6, S4 = 0.2

[a] Initial parameterization as specified by Franks et al. (2014).

The proxy uses stomatal density and pore geometry observed in fossil leaves to compute maximum theoretical stomatal conductance to carbon ($g_{c(max)}$) using diffusion theory (Franks and Beerling, 2009b). However, $g_{c(max)}$ is only one of three components determining total conductance ($g_{c(tot)}$) to CO_2; the other two conductance terms, boundary layer and mesophyll conductance (g_m) are more difficult to calculate directly from fossils. Franks et al. (2014) estimate these unknown terms by prescribing a universal boundary layer resistance of 2 mol m^{-2} s^{-1} and use a multiplier (×0.013) of assimilation rate to estimate g_m. The stable carbon isotopic composition of fossil plant material and that inferred for the atmosphere from marine carbonates are used in the model to compute the ratio of intercellular (c_i) to atmospheric CO_2 concentration (c_i/c_a). This leaves two unknown variables in Eq. (1): (1) the paleo-CO_2 concentration under which the fossil plant developed (c_a) and (2) its assimilation rate (A_n). Fossil plant A_n is estimated by prescribing an initial assimilation rate (A_0) for that fossil under modern ambient CO_2 of 400 ppm (c_{a0}), a photorespiratory CO_2 compensation point (Γ^*) of 40 ppm, and by making the assumption that the relationship between A_n and c_a is universal, has the same slope as the relationship between A_n and c_i, and can be adequately expressed by equations describing the light-limited rate of carbon assimilation. The model then solves for the two unknown variables of interest A_n and c_a by solving Eqs. (1) and (2) simultaneously by iteration.

$$A_n \approx A_0 \left[\frac{(c_a - \Gamma^*)(c_{a0} + 2\Gamma^*)}{(c_a + 2\Gamma^*)(c_{a0} - \Gamma^*)}\right] \quad (2)$$

The new model provides a potentially powerful tool for reconstructing both long- and short-term atmospheric CO_2 and offers a method of inferring paleophysiological function from morphological and isotopic attributes of fossils. Here we attempt to evaluate the performance of the new paleo-CO_2 proxy method of Franks et al. (2014) through a series of sensitivity tests on Paleozoic and Mesozoic fossil plants. Specifically we evaluate how sensitive paleo-CO_2 estimates are to: (1) the physiological constants used in the model; (2) the choice of scaling factors; and (3) initial parameterization of the model.

2. Materials and methods

2.1. Selection of fossil taxa

Six fossil plant taxa, two Devonian, two Carboniferous and two Triassic were selected as test species to evaluate the sensitivity of CO_2 estimates using Franks et al.'s (2014) model to changes in physiological constants, scaling factors and initial model parameterization. Devonian species *Aglaophyton major* and *Asteroxylon mackiei* (Edwards et al., 1998) were used because they have contrasting paleoecophysiology (Wilson and Fischer, 2011) but are coeval. They therefore represent a test of the consistency of paleo-CO_2 estimates from coeval species with inherently different physiology. *Neuropteris ovata* and *Macroneuropteris scheuchzeri*, two common Carboniferous pteridosperms were selected because they possess significantly different stomatal density and geometry (Cleal and Zodrow, 1989) – key inputs of the Franks et al. model – but are likely related phylogenetically and have similar ecological preferences (Stull et al., 2012). They were also used to test

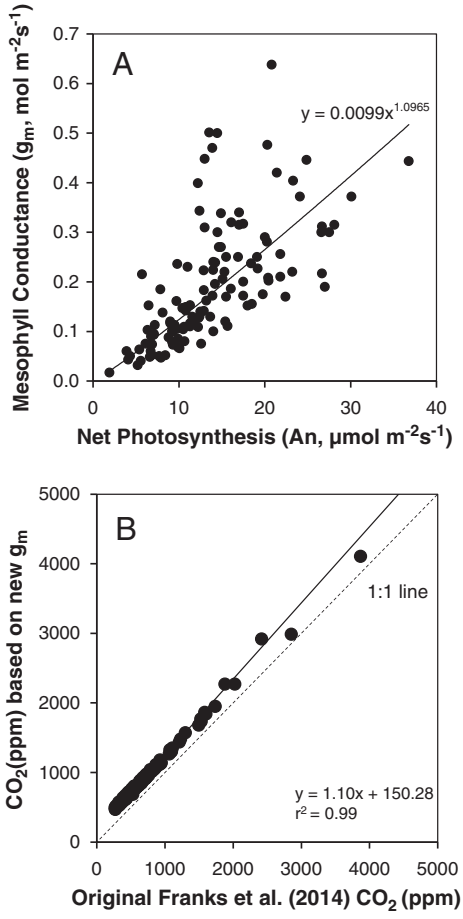

Fig. 1. *A* Relationship between mesophyll conductance (g_m) and net photosynthesis (A_n) for a large extant gymnosperm and angiosperm dataset of (Niinemets et al., 2009) ($r^2 = 0.62$, $n = 122$) used here to derive an estimate of fossil g_m from A_n ($g_m = 0.0099$ $A_n^{1.0965}$). *B* Comparison of pCO_2 estimates from Franks et al. (2014), which uses a simple linear function ($g_m = A_n \times 0.013$) to estimate fossil g_m, with revised pCO_2 estimates where a power function ($g_m = 0.0099$ $A_n^{1.0965}$) from A above has been implemented in the Franks et al. model to estimate g_m. CO_2 estimates with the revised g_m calculations from *A* are consistently 10% greater + 150.28 ppm than in the original Franks et al. model ($y = 1.10x +$ .150.28).

the robustness of the new model under icehouse conditions. Finally, Mesozoic pteridosperms *Dicroidium elongatum* and *D. odontopteroides* (Bomfleur and Kerp, 2010) were selected to test the sensitivity of pCO_2 estimates derived from the stomatal attributes of related taxa under greenhouse conditions.

2.2. Methods

R code to run the paleo-CO_2 model was downloaded and operated according to Franks et al. (2014). As a first test, we evaluated all of the assumptions, constants and scaling factors used. Next, three sensitivity

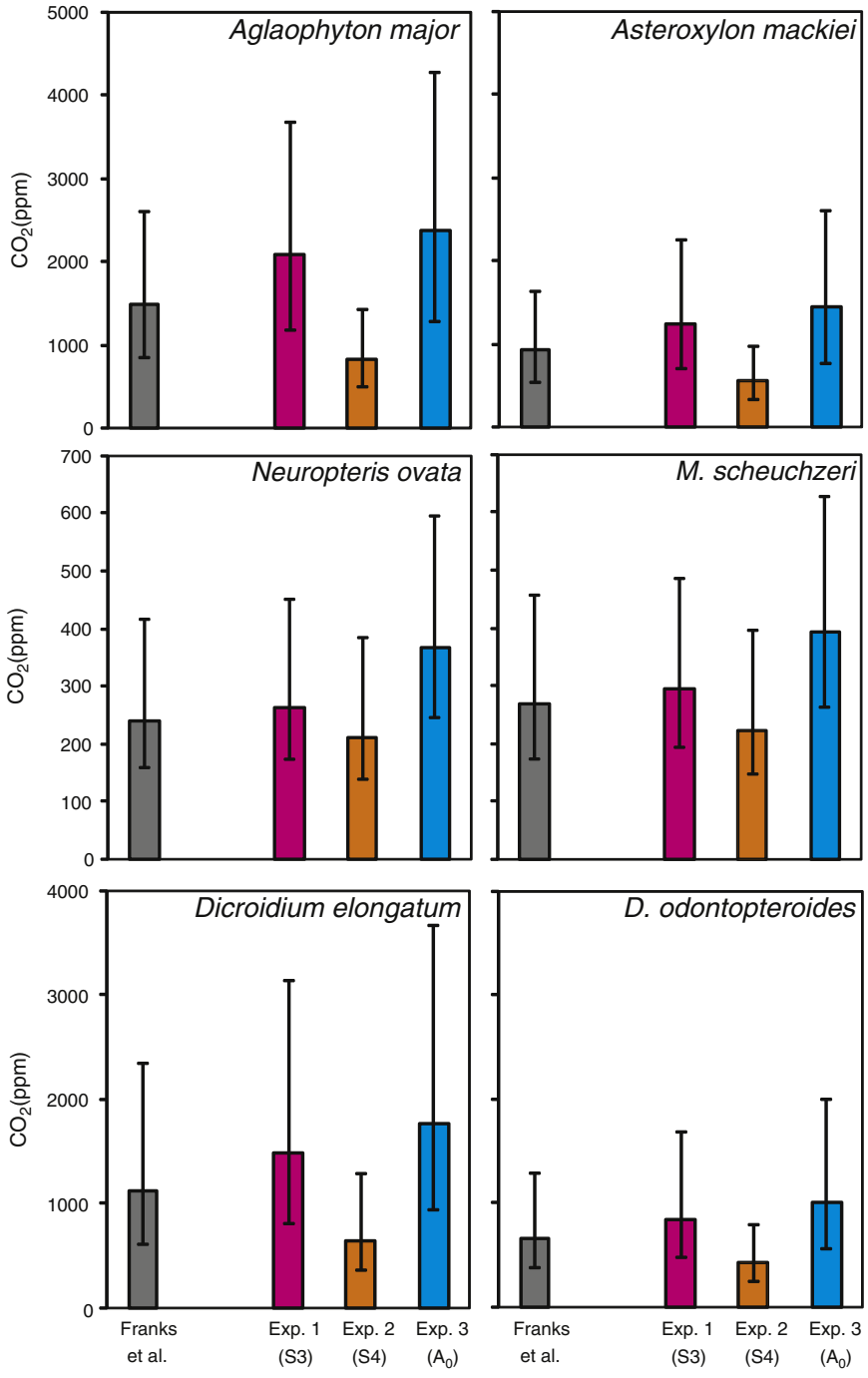

Fig. 2. Sensitivity analysis of Franks et al.'s (2014) paleo-CO_2 proxy model to the scaling factor (S3) used to estimate the geometry of a stomatal pore when fully open (experiment 1) and to the scaling factor (S4) used to estimate what proportion of theoretical maximum rates of conductance a plant typically operates at under field conditions (experiment 2) and to initial parameterization of A_0 (experiment 3). The gray bar of every bar chart 'Franks et al.' indicates CO_2 estimates based on model results using the parameterization and scaling factors reported in Franks et al. (2014) (see Table 1) ($A_0 = 3$ μmol m^{-2} s^{-1} in A and B and 6 μmol m^{-2} s^{-1} in C–F, S3 = 0.6 and S4 = 0.2 for all species). The pink bars indicate CO_2 estimates based on results from experiment 1 where the scaling factor 'S3' was changed from 0.6 to 0.4 to simulate fossil plants which were capable of maximally opening their stomatal pores to 40% of a full circle instead of 60%. All other initial conditions and scaling factors were kept the same as in Franks et al. The orange bars indicate CO_2 estimates based on model results from experiment 2 where the scaling factor 'S4' was changed from 0.2 to 0.4 to simulate fossil plants operating at 40% of their maximum theoretical potential $g_{c(max)}$ rather than 20%. Blue bars indicate CO_2 estimates based on model results from experiment 3 where parameterization of A_0 was changed according to Table 1 (Exp. 3) and all other initial conditions and scaling factors were kept the same as in Franks et al. All other initial conditions and scaling factors were kept the same as in Franks et al. The error bars represent the 16th and 84th percentile bootstrapped errors computed by the model.

experiments were undertaken on the six selected fossil species. Experiment 1 investigated sensitivity to changes in the scaling factor (S3) used to estimate the geometry of a stomatal pore when fully open from measurements of fossil pore length. Here the scaling factor S3 was changed from 0.6 to 0.4 to simulate fossil plants which opened their stomatal pores to 40% of a full circle instead of 60% (prescribed by Franks et al., 2014), in line with observations of Weyers and Lawson (1997). All other initial conditions and scaling factors were kept the same as in Franks et al. (Table 1 Exp. 1). Experiment 2 tested the sensitivity of proxy CO_2 reconstruction to changes in the scaling factor (S4) used to estimate the proportion of theoretical maximum rates of stomata conductance ($g_{c(max)}$) a plant typically operates at under field conditions. The scaling factor S4 was changed from 0.2 (Franks et al. 2014) to 0.4 to simulate fossil plants operating at 40% of their maximum theoretical potential $g_{c(max)}$ rather than only 20% following observations of maximum operational conductance for some modern taxa in McElwain et al. (2015) (Table 1 Exp. 2). Experiment 3 investigated the sensitivity of paleo-CO_2 estimates to changes in the initial parameterization of fossil plant assimilation rate (A_0). Following a control run which implemented all the recommended initial conditions, scaling factors and A_0 values used in Franks et al. (Table 1 control), the model was re-run with modified A_0 values only (Table 1 Exp. 3).

3. Evaluation of a new proxy-atmospheric CO_2 model

3.1. Initial assessment of constants and scaling factors

The leaf internal CO_2 concentration at which a plant's net assimilation rate equals CO_2 release through the process of photorespiration (in the absence of dark respiration) is known as the photorespiratory compensation point. This compensation point (Γ^* in Eq. (2)) is temperature, species and O_2 dependent (Ethier and Livingston, 2004) but Franks et al. (2014) account only for the temperature dependency in the new paleo-CO_2 proxy model. Allowing Γ^* to vary in response to prevailing paleoatmospheric O_2 concentration [O_2] ($\Gamma^* = 1.78 \times [O_2]$), which is known to have varied widely (10% to 30%) through the Phanerozoic (Bergman et al., 2004; Belcher and McElwain, 2008; Berner, 2009), would increase the precision of paleo-CO_2 estimates but only fractionally.

The model also down weights the importance of species disparity in mesophyll conductance (g_m) (Flexas et al., 2012; Flexas et al., 2014) and uses a simple linear function ($g_m = A_n \times 0.013$) to estimate g_m of fossils from their photosynthetic rate (A_n). Although g_m cannot be measured directly from fossil plant material it is possible to estimate g_m from leaf anatomical traits such as mesophyll cell wall thickness (Tomás et al., 2013). We have attempted to account for wider variation in g_m over geological time by using a power function to estimate fossil g_m from A_n ($g_m = 0.0099\ A_n^{1.0965}$) derived from the best fit relationship for a large extant gymnosperm and angiosperm dataset of Niinemets et al. (2009) ($r^2 = 0.62$, $n = 122$) (Fig. 1A). The comparison suggests that oversimplification in the treatment of the passage of CO_2 and H_2O through mesophyll leaf tissue (g_m) in the Franks et al. model has resulted in an underestimation of paleo-CO_2 values by 10% plus an overall 150 ppm for all taxa (Fig. 1B). This is demonstrated by the 10% plus 150.28 ppm offset from unity in the regression line between Franks' CO_2 (x axis) and our revised CO_2 estimates (y axis) in Fig. 1B.

The new model is also highly sensitive to scaling factor S3 used to estimate maximum opening of a fossil stoma from guard cell anatomical measurements (Exp. 1 in Fig. 2) and scaling factor S4 used to estimate operational conductance from maximum theoretical stomatal conductance $g_{c(max)}$ (Exp. 2 in Fig. 2). The scaling factor S3 is needed because living plants have been shown to have different stomatal pore geometries when the pore is fully open and rarely in fossils are stomata preserved open in full operating position. An estimate is required therefore to scale between the maximum opening geometry of a fossil stomatal pore (e.g. a circle with diameter equal to stomatal pore length) and the actual geometry when operating at maximum capacity (e.g. some proportion of a circle). Results from sensitivity experiment 1 (Fig. 2) demonstrate that paleo-atmospheric CO_2 estimates derived from fossil stomata using the Franks et al. (2014) model are substantially higher for some taxa (e.g. +595 ppm higher in *Aglaophyton*) but negligibly higher in others (e.g. +22 ppm higher in *Neuropteris*) if it is assumed that fossil taxa open their stomata to a maximum of 40% of a circle instead of 60% as set by Franks et al. (2014). Uncertainty in initial parameterization of S3 will therefore only propagate large uncertainty in paleo-CO_2 estimates for fossil taxa with inherently low stomatal conductance, that is those with few very large stomata (e.g. *Aglaophyton*), and much less so for taxa with high conductance resulting from a high density of small stomata (e.g. *Neuropteris*).

Results from experiment 2 highlight the potential error in pCO_2 estimates that result from uncertainty in estimating how typical day-to-day operation of a plant's gas exchange ($g_{c(op)}$) relates to the maximum theoretical capacity ($g_{c(max)}$) of that plant to conduct CO_2 through stomata. Franks et al. (2014) have assumed that all fossil taxa were conducting CO_2 through their stomata at 20% of their theoretical maximum potential. Based on available data this was a fair initial assumption. However, it has recently been demonstrated that the scaling relationship between operational and anatomically derived maximum stomatal conductance (S4) is CO_2 dependent (Dow et al., 2014) and can be highly species specific (McElwain et al., 2015). The results presented here (Fig. 2) demonstrate a subtle reduction in paleo-CO_2 estimates for all species investigated if it is assumed that plants operate at 40% rather than only 20% of their theoretical maximum limits (Exp. 2 in Fig. 2). Uncertainty in initial parameterization of S4 in the range of +/− 10% appear therefore to be well accounted for in the uncertainty propagation of Franks et al. (2014). Our analysis indicates that the model is most sensitive to initial parameterization of fossil plants assimilation rate (A_0) which we elaborate on in more detail below.

3.2. Parameterizing assimilation rates of fossil plants

The new paleo-CO_2 proxy (Franks et al., 2014) requires that an initial assimilation rate (A_0) at a CO_2 concentration (c_{a0}) of 400 ppm, is

Table 2
Results from approach to estimate assimilation rate (A_0) from fossil leaf morphological traits such as vein density (D_v) and maximum stomatal conductance to water vapor ($g_{w(max)}$) following McElwain et al. (2015).

Fossil taxon	D_v[a] mm mm^{-2}	g_{wmax} mmol H_2O m^{-2} s^{-1}	Equation to estimate A_0 from D_v and g_{max}[b]	Estimated A_0 μmol CO_2 mm^{-2} s^{-1}
Neuropteris ovata	4.49	2259	$A_0 = 3.1225.\log_e(g_{max})–8.18$ (for taxa with $D_v > 2 < 5$ mm mm^{-2})	15.9
Macroneuropteris scheuchzeri	3.42	1127	$A_0 = 3.1225.\log_e(g_{max})–8.18$ (for taxa with $D_v > 2 < 5$ mm mm^{-2})	13.7
Dicroidium elongatum	<2.0	1554	$A_0 = 1.9624.\log_e(g_{max})–4.06$ (for taxa with $D_v < 2$ mm mm^{-2})	10.3
Dicroidium odontopteroides	1.63	1782	$A_0 = 1.9624.\log_e(g_{max})–4.06$ (for taxa with $D_v < 2$ mm mm^{-2})	10.6

[a] D_v data from Boyce and Zwieniecki (2012).
[b] Equations from McElwain et al. (2015).

parameterized for each fossil plant species used to reconstruct paleo-CO_2. On first consideration this seems like an impossible task, especially when considering extinct lineages such as Rhyniophytes (most basal land plants), pteridosperms and Bennettitales (some early seed plant lineages), which all lack modern ecophysiological analogues to guide parameterization of A_0. Franks et al. solved the initial challenge by taking a standardized approach, assigning A_0 values of 3 μmol m^{-2} s^{-1} to all spore bearing lineages (Rhyniophytes, Lycophytes, Sphenophytes), 6 μmol m^{-2} s^{-1} to all non-coniferous gymnosperms (pteridosperms, cycads, Bennettitales), 10 μmol m^{-2} s^{-1} to conifers and 12 μmol m^{-2} s^{-1} to all angiosperms. This is a useful initial approach. However given the high sensitivity of the paleoproxy model to parameterization of A_0 demonstrated in Fig. 2 (Exp. 3), improved characterization of fossil plant assimilation is needed before the full potential of the new method can be realized.

For example, assigning A_0 values of 6 μmol m^{-2} s^{-1} instead of 3 μmol m^{-2} s^{-1} to *Aglaophyton major* and *Asteroxylon mackiei* (Edwards et al., 1998) increased the paleo-CO_2 estimate by +870 ppm and +505 ppm respectively for the early Devonian. Estimates of early Devonian CO_2 are therefore largely dependent on the initial assimilation rates parameterized for each taxon. While the lower range of bryophyte assimilation rates (*e.g.* 2–3 μmol m^{-2} s^{-1}) may be appropriate for a non-vascular plant such as *Aglaophyton*, a full-sun tropical lycophyte analogue with values ranging between 5 and 6 μmol m^{-2} s^{-1} (Brodribb and Holbrook, 2006) or higher may be more appropriate for the vascular basal lycophyte *Asteroxylon mackiei*, which had high hydraulic conductivity (Wilson and Fischer, 2011). Notably, when more appropriate A_0 values are chosen for these two contrasting fossil taxa, the paleo-CO_2 estimates for the early Devonian derived from Franks et al.'s model converge (Fig. 2 'Franks' model run for *Aglaophyton* (gray bar) vs. Exp. 3 model run for Asteroxylon (blue bar)), whereas when both were parameterized with the same A_0 values the disparity in predicted CO_2 concentration was 500 ppm.

Although it is difficult to confidently choose appropriate living physiological analogues for extinct plants, A_0 values could be estimated using published scaling relationships (for extant taxa) between morphological variables, which influence the movement of water and/or CO_2 in the leaf (and stem in the case of water), such as vein density (D_v), vein to stomatal distance (D_m), maximum theoretical conductance to water ($g_{w(max)}$) and maximum assimilation rate (A_{max}) (Brodribb et al., 2007; Brodribb and Feild, 2010; Boyce and Zwieniecki, 2012; McElwain et al., 2015; Wilson et al., 2015). As a test of this approach we used published vein density measurements (Boyce and Zwieniecki, 2012) for *N. ovata*, *M. scheuchzeri*, *D. elongatum* and *D. odontopteroides* together with their calculated $g_{w(max)}$ values derived from stomatal density and pore geometry measurements (Bomfleur and Kerp, 2010; Wilson et al., 2015) to estimate A_0 using scaling relationships established between A_0, D_v and $g_{w(max)}$ for extant taxa in McElwain et al. (2015) (Table 2).

As an additional check on the approach of using D_v and $g_{w(max)}$ to estimate A_0 we also employed established scaling relationships between the anatomical trait D_m, which is a measure of the shortest distance between leaf veins and stomata, leaf hydraulic capacity (K_{leaf}), and maximum photosynthetic rate (A_{max}) (Brodribb et al., 2007) for *N. ovata*. Short distances between leaf veins and stomata (D_m) increase K_{leaf} and also maximal photosynthetic rate (A_{max}), whereas leaves with fewer veins and/or longer pathways for mesophyll conductance have lower K_{leaf} and, consequently, lower A_{max} values. D_m was measured from transverse sections of well-preserved fossil plant leaves, including collected leaves and previously published images (Beeler, 1983). To be conservative, taxon-average D_m values were rounded up to the next-highest 25 microns. K_{leaf} was calculated from D_m using the following equation:

$$K_{leaf} = 12775 * (D_m)^{-1.26}$$

A_{max} was calculated from K_{leaf} using a polynomial fit to the observed scaling relationship for plants that lack specialized mesophyll conducting fibers (i.e., transfusion tissue), using the following equation:

$$A_{max} = -0.022 * (K_{leaf})^2 + 1.320 * (K_{leaf}) - 0.261$$

Because this equation relates anatomical measurements to maximal photosynthetic rates that were experimentally measured at 370 ppm CO_2, A_{max} and A_0 are nearly identical parameters, despite being derived from different scaling relationships. Very good congruence in A_0 estimates was achieved for *N. ovata* from the two alternative methods using either morphological (Table 2) or anatomical (Table 3) traits lending confidence to our overall approach.

The resultant A_0 estimates for *N. ovata* (A_0 = 16 μmol m^{-2} s^{-1}), *M. scheuchzerii* (A_0 = 13 μmol m^{-2} s^{-1}), *D. elongatum* (A_0 = 10 μmol m^{-2} s^{-1}) and *D. odontopteroides* (A_0 = 10 μmol m^{-2} s^{-1}) (Tables 2 and 3) are all substantially higher than the standardized parameterization of A_0 (6 μmol m^{-2} s^{-1}) used in Franks et al. (2014). We then compared Franks et al.'s paleo-CO_2 estimates where all 4 pteridosperms were parameterized with A_0 values of 6 μmol m^{-2} s^{-1} (Fig. 2 'Franks') with our revised paleo-CO_2 estimates after re-parameterization of A_0 with species appropriate values (Tables 2 and 3; Fig. 2 'Exp. 3'). The resultant paleo-CO_2 estimates increased by up to 646 ppm for the Mesozoic and by *c.* 130 ppm for the Carboniferous based on these examples.

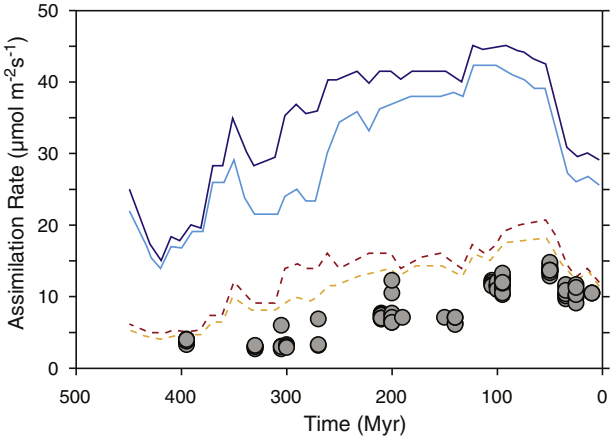

Fig. 3. Simulated changes in net assimilation rate over geological time using the Sheffield Dynamic Vegetation Model (solid and dashed lines indicating upper and lower bound estimates respectively; dark blue and dashed red show model results under paleo-conditions of fixed temperature and atmospheric O_2, light blue and dashed orange show model results under variable paleo-temperature and atmospheric O_2) from Franks and Beerling (2009a) compared with maximum assimilation rate estimates (gray circles) derived from the new paleoproxy CO_2 model (Franks et al., 2014). Note that over half of the individual point estimates from Franks et al. (2014) are well under the 'lower bound' estimates of Franks and Beerling (2009a) suggesting that initial parameterization of A_0 in Franks et al. (2014) may be too low resulting in underestimation of CO_2 concentrations for large parts of the late Paleozoic and Mesozoic.

Table 3
Results from approach to estimate assimilation rate (A_0) from fossil plant anatomical traits (D_m) and K_{leaf} for *Neuropteris ovata*.

Taxon	Path length (D_m; μm)	K_{leaf} (mmol H_2O/(m^{-2} s^{-1} MPa))	Estimated A_0 μmol CO_2 mm^{-2} s^{-1}
Neuropteris ovata	200	16.11	15.29

The sensitivity analysis of Franks' model to A_0 suggests that the authors claim that post-Paleozoic CO_2 was capped in the long-term below 1000 ppm may be premature. We have shown that many of the Mesozoic pteridosperms used for input data to run Franks et al.'s model were assigned with A_0 values that are likely too low (some by up to 100%) and which cannot be justified based on their preserved morphological and anatomical traits. Re-parameterization of A_0 upwards using fossil vein density (D_v) data (Boyce and Zwieniecki, 2012) and published relationships between D_v and/or D_m with A_0 for extant plants (Brodribb et al., 2007; Brodribb and Feild, 2010; Boyce and Zwieniecki, 2012) or between D_v, $g_{w(max)}$ and A_0 (McElwain et al., 2015) would raise Mesozoic paleo-CO_2 estimates well above 1000 ppm. This re-parameterization would also bring fossil plant assimilation rates (A_n) calculated as a by-product of the new paleo-CO_2 proxy model (Franks et al., 2014) into line with existing (Franks and Beerling, 2009a) estimates of assimilation changes over geological time (Fig. 3).

4. Concluding remarks

Despite the uncertainties discussed, our analysis highlights that the new mechanistic paleo-CO_2 proxy of Franks et al. (2014) has significant potential to derive robust and more accurate CO_2 estimates from fossil plant stomata, as long as parameterization of initial conditions in the model, particularly A_0, are strongly justified with supporting morphological and/or anatomical data. It also reveals the potential power of the model to better constrain differences in paleophysiology of cohabiting or coeval fossil species (e.g. *Aglaophyton* and *Asteroxylon*). A perhaps unexpected outcome of the new proxy is that it brings into sharp focus the need to improve understanding of how productivity of Earth's vegetation has changed over time through better estimates of fossil plant carbon assimilation rates from morphological and anatomical traits such as vein density, vein-to-stomatal path length and g_{max} and through a more detailed understanding of how well anatomical g_{max} computed from fossils can predict the actual stomatal conductance of fossil taxa when they were living.

Acknowledgements

We thank the European Research Council (ERC-StG-279962) and the National Science Foundation (NSF-ELT-1338281) for funding. G. Griggs (UC Davis) is thanked for assistance with this project in summer 2014.

References

Barclay, R.S., McElwain, J.C., Sageman, B.B., 2010. Carbon sequestration activated by a volcanic CO_2 pulse during Ocean Anoxic Event 2. Nat. Geosci. 3, 205–208.
Beeler, H.E., 1983. Anatomy and frond architecture of *Neuropteris ovata* and *Neuropteris scheuchzeri* from the Upper Pennsylvanian of the Appalachian Basin. Can. J. Bot. 61, 2352–2368.
Belcher, C.M., McElwain, J.C., 2008. Limits for combustion in low O_2 redefine paleoatmospheric predictions for the Mesozoic. Science 321, 1197–1200.
Bergman, N.M., Lenton, T.M., Watson, A.J., 2004. COPSE: a new model of biogeochemical cycling over Phanerozoic time. Am. J. Sci. 304, 397–437.
Berner, R., 1990. Atmospheric carbon dioxide levels over Phanerozoic time. Science 249, 1382–1386.
Berner, R.A., 2006. GEOCARBSULF: a combined model for Phanerozoic atmospheric O_2 and CO_2. Geochim. Cosmochim. Acta 70, 5653–5664.
Berner, R.A., 2009. Phanerozoic atmospheric oxygen: new results using the Geocarbsulf model. Am. J. Sci. 309, 603–606.
Bomfleur, B., Kerp, H., 2010. *Dicroidium* diversity in the Upper Triassic of north Victoria Land, East Antarctica. Rev. Palaeobot. Palynol. 160, 67–101.
Boyce, C.K., Zwieniecki, M.A., 2012. Leaf fossil record suggests limited influence of atmospheric CO_2 on terrestrial productivity prior to angiosperm evolution. Proc. Natl. Acad. Sci. U. S. A. 109, 10403–10408.
Breecker, D., Sharp, Z., McFadden, L., 2010. Atmospheric CO_2 concentrations during ancient greenhouse climates were similar to those predicted for AD 2100. Proc. Natl. Acad. Sci. 107, 576–580.
Brodribb, T.J., Feild, T.S., 2010. Leaf hydraulic evolution led a surge in leaf photosynthetic capacity during early angiosperm diversification. Ecol. Lett. 13, 175–183.
Brodribb, T.J., Holbrook, N.M., 2006. Declining hydraulic efficiency as transpiring leaves desiccate: two types of response. Plant Cell Environ. 29, 2205–2215.
Brodribb, T.J., Feild, T.S., Jordan, G.J., 2007. Leaf maximum photosynthetic rate and venation are linked by hydraulics. Plant Physiol. 144, 1890–1898.
Cleal, C.J., Zodrow, E.L., 1989. Epidermal structure of some medullosan *Neuropteris* foliage from the Middle and Upper Carboniferous of Canada and Germany. Palaeontology 32, 837–882.
Dow, G.J., Bergmann, D.C., Berry, J.A., 2014. An integrated model of stomatal development and leaf physiology. New Phyt. 201, 1218–1226.
Edwards, D., Kerp, H., Hass, H., 1998. Stomata in early land plants: an anatomical and ecophysiological approach. J. Exp. Bot. 49, 255–278.
Ekart, D.D., Cerling, T.E., Montañez, I.P., Tabor, N.J., 1999. A 400 million year carbon isotope record of pedogenic carbonate: implications for paleoatmospheric carbon dioxide. Am. J. Sci. 299, 805–827.
Ethier, G., Livingston, N., 2004. On the need to incorporate sensitivity to CO_2 transfer conductance into the Farquhar–von Caemmerer–Berry leaf photosynthesis model. Plant Cell Environ. 27, 137–153.
Farquhar, G.D., Sharkey, T.D., 1982. Stomatal conductance and photosynthesis. Annu. Rev. Plant Physiol. 33, 317–345.
Flexas, J., Barbour, M.M., Brendel, O., Cabrera, H.M., Carriquí, M., Díaz-Espejo, A., Douthe, C., Dreyer, E., Ferrio, J.P., Gago, J., 2012. Mesophyll diffusion conductance to CO_2: an unappreciated central player in photosynthesis. Plant Sci. 193, 70–84.
Flexas, J., Carriquí, M., Coopman, R.E., Gago, J., Galmés, J., Martorell, S., Morales, F., Diaz-Espejo, A., 2014. Stomatal and mesophyll conductances to CO_2 in different plant groups: underrated factors for predicting leaf photosynthesis responses to climate change? Plant Sci. 226, 41–48.
Franks, P.J., Beerling, D.J., 2009a. CO_2-forced evolution of plant gas exchange capacity and water-use efficiency over the Phanerozoic. Geobiology 7, 227–236.
Franks, P.J., Beerling, D.J., 2009b. Maximum leaf conductance driven by CO_2 effects on stomatal size and density over geologic time. Proc. Natl. Acad. Sci. U. S. A. 106, 10343–10347.
Franks, P.J., Royer, D.L., Beerling, D.J., VandeWater, P.K., Cantrill, D.J., Barbour, M.M., Berry, J.A., 2014. New constraints on atmospheric CO_2 concentration for the Phanerozoic. Geophys. Res. Lett. 41, 4685–4694.
Hansen, J., Sato, M., Kharecha, P., Beerling, D., Berner, R., Masson-Delmotte, V., Pagani, M., Raymo, M., Royer, D.L., Zachos, J.C., 2008. Target atmospheric CO_2: where should humanity aim? Open Atmos. Sci. J. 2, 217–231.
McElwain, J.C., Yiotis, C., Lawson, T., 2015. Using modern plant trait relationships between observed and theoretical maximum stomatal conductance and vein density to examine patterns of plant macroevolution. New Phytol. http://dx.doi.org/10.1111/nph.13579.
Montañez, I., Soreghan, G.S., 2006. Earth's fickle climate: lessons learned from deep-time ice ages. Geotimes 51, 24–27.
Niinemets, Ü., Díaz-Espejo, A., Flexas, J., Galmés, J., Warren, C.R., 2009. Role of mesophyll diffusion conductance in constraining potential photosynthetic productivity in the field. J. Exp. Bot. 60, 2249–2270.
Pagani, M., Zachos, J.C., Freeman, K.H., Tipple, B., Bohaty, S., 2005. Marked decline in atmospheric carbon dioxide concentrations during the Paleogene. Science 309, 600–603.
Royer, D.L., Berner, R.A., Montanez, I.P., Tabor, N.J., Beerling, D.J., 2004. CO_2 as a primary driver of Phanerozoic climate. GSA Today 14, 4–10.
Schubert, B.A., Jahren, A.H., 2012. The effect of atmospheric CO_2 concentration on carbon isotope fractionation in C3 land plants. Geochim. Cosmochim. Acta 96, 29–43.
Steinthorsdottir, M., Jeram, A.J., McElwain, J.C., 2011. Extremely elevated CO_2 concentrations at the Triassic/Jurassic boundary. Palaeogeogr. Palaeoclimatol. Palaeoecol. 308, 418–432.
Stull, G.W., DiMichele, W.A., Falcon-Lang, H.J., Nelson, W.J., Elrick, S., 2012. Palaeoecology of *Macroneuropteris scheuchzeri*, and its implications for resolving the paradox of 'xeromorphic' plants in Pennsylvanian wetlands. Palaeogeogr. Palaeoclimatol. Palaeoecol. 331, 162–176.
Tomás, M., Flexas, J., Copolovici, L., Galmés, J., Hallik, L., Medrano, H., Ribas-Carbó, M., Tosens, T., Vislap, V., Niinemets, Ü., 2013. Importance of leaf anatomy in determining mesophyll diffusion conductance to CO_2 across species: quantitative limitations and scaling up by models. J. Exp. Bot. 64, 2269–2281.
Von Caemmerer, S., 2000. Biochemical models of leaf photosynthesis. CSIRO Publishing, Collingwood, Victoria, Australia.
Weyers, J.D.B., Lawson, T., 1997. Heterogeneity in stomatal characteristics. Advan. in Bot. Res. 26, 317–352.
Wilson, J.P., Fischer, W.W., 2011. Hydraulics of *Asteroxylon mackei*, an early Devonian vascular plant, and the early evolution of water transport tissue in terrestrial plants. Geobiology 9, 121–130.
Wilson, J.P., White, J.D., DiMichele, W.A., Hren, M.T., Poulsen, C.J., McElwain, J.C., Montañez, I.P., 2015. Reconstructing extinct plant water use for understanding vegetation-climate feedbacks: methods, synthesis and a case study using the Paleozoic era medullosan seed ferns. Paleontol. Soc. Pap. 21, 167–195.