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<td><strong>Authors(s)</strong></td>
<td>Yiotis, Charilaos, Evans-FitzGerald, Christiana, McElwain, Jennifer C.</td>
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<td><strong>Publication date</strong></td>
<td>2017-03-11</td>
</tr>
<tr>
<td><strong>Publisher</strong></td>
<td>Oxford University Press</td>
</tr>
<tr>
<td><strong>Item record/more information</strong></td>
<td><a href="http://hdl.handle.net/10197/8504">http://hdl.handle.net/10197/8504</a></td>
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<tr>
<td><strong>Publisher’s statement</strong></td>
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<tr>
<td><strong>Publisher’s version (DOI)</strong></td>
<td>10.1093/aob/mcx018</td>
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Differences in the photosynthetic plasticity of ferns and *Ginkgo* grown in experimentally controlled low [O$_2$]:[CO$_2$] atmospheres may explain their contrasting ecological fate across the Triassic–Jurassic mass extinction boundary

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Received: 4 October 2016 Returned for revision: 19 December 2016 Editorial decision: 20 January 2017 Accepted: 1 February 2017

**Background and Aims** Fluctuations in [CO$_2$] have been widely studied as a potential driver of plant evolution; however, the role of a fluctuating [O$_2$]:[CO$_2$] ratio is often overlooked. The present study aimed to investigate the inherent physiological plasticity of early diverging, extant species following acclimation to an atmosphere similar to that across the Triassic–Jurassic mass extinction interval (TJB, approx. 200 Mya), a time of major ecological change.

**Methods** Mature plants from two angiosperm (*Drimys winteri* and *Chloranthus oldhamii*), two monilophyte (*Osmunda claytoniana* and *Cyatheus australis*) and one gymnosperm (*Ginkgo biloba*) species were grown for 2 months in replicated walk-in Conviron BDW40 chambers running at TJB treatment conditions of 16 % [O$_2$]–1900 ppm [CO$_2$] and ambient conditions of 21 % [O$_2$]–400 ppm [CO$_2$], and their physiological plasticity was assessed using gas exchange and chlorophyll fluorescence methods.

**Key Results** TJB acclimation caused significant reductions in the maximum rate of carboxylation ($V_{Cmax}$) and the maximum electron flow supporting ribulose-1,5-bisphosphate regeneration ($J_{max}$) in all species, yet this downregulation had little effect on their light-saturated photosynthetic rate ($A_{sat}$). *Ginkgo* was found to photorespire heavily under ambient conditions, while growth in low [O$_2$]:[CO$_2$] resulted in increased heat dissipation per reaction centre ($DI/RC$), severe photodamage, as revealed by the species’ decreased maximum efficiency of primary photochemistry ($F_{v}/F_{m}$) and decreased *in situ* photosynthetic electron flow ($J_{sw}$).

**Conclusions** It is argued that the observed photodamage reflects the inability of *Ginkgo* to divert excess photosynthetic electron flow to sinks other than the downregulated C$_3$ and the diminished C$_2$ cycles under low [O$_2$]:[CO$_2$]. This finding, coupled with the remarkable physiological plasticity of the ferns, provides insights into the underlying mechanism of Ginkgoales’ near extinction and ferns’ proliferation as atmospheric [CO$_2$] increased to maximum levels across the TJB.

**Key words:** Triassic–Jurassic boundary, *Ginkgo biloba*, gymnosperms, monilophytes, angiosperms, high CO$_2$, low O$_2$, photosynthetic plasticity, photorespiration, photodamage, stomatal conductance, mesophyll conductance.

**INTRODUCTION**

The end of the Triassic marked the beginning of a period of geological and ecological upheaval known as the Triassic–Jurassic mass extinction event (approx. 200 Mya). Although several studies have questioned the high rates (Hallam, 2002; Tanner et al., 2004) and sources (Bambach et al., 2004) of biodiversity loss across the Triassic–Jurassic boundary (TJB), it is widely considered as the third greatest mass extinction in the Phanerozoic (last approx. 450 million years) (Benton, 1995; McElwain and Punyasena, 2007). Plant communities were severely affected in terms of species turnover rates (Harris, 1937; Visscher and Brugman, 1981; Fowel et al., 1994; McElwain et al., 1999, 2007, 2009; Olsen et al., 2002) and evenness (i.e. the equality of relative abundances among taxa, McElwain et al., 2007, 2009), yet the available data suggest that higher taxonomic ranks displayed remarkable resilience (Ash, 1986; McElwain et al., 2007; Willis and McElwain, 2013). In general, there is a negligible impact of mass extinctions on plants at the family level (Ash, 1986; McElwain et al., 2007; McElwain and Punyasena, 2007). Instead, plant communities undergo structural reformation, which includes substantial changes of families’ relative abundances and distributions and/or in some cases the total loss of growth habits (McElwain et al., 2007, 2009; McElwain and Punyasena, 2007; Bonis and Kuerschner, 2012). In this context, differences in the physiological plasticity between species, families or reproductive groups are expected to play a role in shaping the composition of plant communities under changing environmental conditions.

The causal mechanism of the TJB mass extinction has been actively debated (Hallam, 1997; McElwain et al., 1999, 2009; Palfy et al., 2001; Beerling and Berner, 2002; Hesselbo et al., 2002; Olsen et al., 2002; Marzoli et al., 2004). The organic carbon isotope record revealed a major, and synchronous with the mass extinction event, perturbation of the global carbon cycle in the form of a light carbon excursion across the TJB.
The oxygenation/carboxylation activity of plants’ primary carboxylase (i.e. Rubisco), every change in the relative abundance of [O2] and [CO2] in the atmospheric mixture results in corresponding changes in the enzyme’s carboxylation and oxygenation rates, which are accompanied by dynamic adjustments of the energy flows that support them with ATP and reducing power in the form of NADPH (Farquhar et al., 1980; von Caemmerer, 2000). Since all the light energy absorbed by a plant needs to be quenched, a fine balance between the energy and the reducing power produced during the ‘light reactions’ and those consumed at the ‘dark reactions’ of photosynthesis and photorespiration is essential (Sharkey, 1990; Zhang and Portis, 1999; Andersson, 2008; Parry et al., 2008). However, acclimation to high CO2 is known to result in a wide range of morphological and physiological adaptations such as the down-regulation of Rubisco activity and an increase in the resistances to CO2 diffusion (Ainsworth and Long, 2005; Kirkham, 2011; Kitao et al., 2015) that further complicate the necessary fine adjustment of the energy flows within the photosynthetic machinery.

Accordingly, the aim of the present study was to address this gap in our knowledge by investigating the responses of plants belonging to all three major plant reproductive grades (angiosperms, gymnosperms and ferns) after exposure to O2 and CO2 atmospheric concentrations similar to those that prevailed across the TJB. Our hypothesis was that the enhanced resilience of ferns across the TJB low [O2];[CO2] bottleneck was, at least partly, a result of their inherent increased physiological plasticity under low [O2];[CO2] conditions compared with Ginkgo. In particular, our study focused on the ability of the different plant groups to readjust the energy flows within their photosynthetic apparatus effectively so that they match the acclimated rates of Rubisco carboxylation and oxygenation.

Our approach did not exclude the angiosperms, even though the general consensus is that they first evolved much later than the TJB, during the Cretaceous (Crane et al., 1995; Soltis and Soltis, 2004). A combined consideration of the generic diversity in angiosperms (Niklas et al., 1983) and the atmospheric levels of O2 and CO2 (Berner, 2006) reveals that their explosive radiation and final dominance was seemingly unaffected by the rapid increase in the initially low, and similar to that of the TJB, [O2];[CO2] ratio. Based on their evolutionary history and ecological success through a wide range of [O2];[CO2] ratios, we anticipated that the angiosperms would also display an increased plasticity compared with Ginkgo under simulated low [O2];[CO2] atmospheric conditions.

Materials and Methods

Plant material

Mature plants from five species belonging to some of the most early diverging families within each major reproductive grade (Supplementary Data Table S1), namely the monophytes Osmunda claytoniana L. and Cyathea australis (R. Brown) Domín, 1929, the gymnosperm, ‘living fossil’ Ginkgo biloba L., and the angiosperm species Drimys winteri J.R. Forst. & G. Forst and Chloranthus oldhamii Solms were purchased, repotted and then acclimated to glasshouse conditions (mean temperature = 18°C) at University College Dublin Rosemount Environmental Research Station glasshouses for approx. 2 weeks. Pot size, soil mixture, type and amount of fertilizer used were determined independently based on the size, age and special preferences of each species (Table S1).
Controlled-environment experiments

A trial study to assess potential ‘chamber effects’ was conducted prior to the initiation of growth chamber experiments (Porter et al., 2015). The study revealed that some of the eight Conviron BDW40 (Winnipeg, Manitoba, Canada) walk-in growth chambers of University College Dublin’s PEAC facility (Rosemount, University College Dublin) display significant ‘chamber effects’, thus their further use in the present study was avoided. Following the 2 week acclimation under glass-house conditions, 2–3 fully expanded leaves were tagged on each individual and the plants were then transferred into four BDW40 growth chambers that displayed no chamber effects (see Porter et al., 2015). Half of the plants were subjected to a replicated TJB atmospheric treatment in two of the chambers for 2 months, while the rest were grown under ambient atmospheric conditions for the same amount of time, serving as our controls (Supplementary Data Table S2). The atmospheric composition used for the TJB treatment was 16 % O₂ and 1900 ppm CO₂, which is a good approximation of the corresponding mean values for atmospheric O₂ and CO₂ across the boundary reported by mass balance modelling and palaeo-proxy studies (McElwain et al., 1999; Berner, 2001, 2006; Bergman et al., 2004; Belcher and McElwain, 2008; Bonis et al., 2010; Steinthorsdottir et al., 2011). The O₂ and CO₂ concentrations used for the ambient treatment were 21 % and 400 ppm, respectively (Table S1).

CO₂ in each chamber was monitored by a WMA-4 infrared analyser (PP-Systems, Amesbury, MA, USA), and injection of compressed CO₂ (BOC Gases Ireland Ltd, Bluebell, County Dublin, Ireland) enabled stable within-chamber CO₂ concentrations well above ambient levels. The O₂ concentration in each chamber was monitored by a PP-systems OP-1 oxygen sensor, and injection of compressed N₂ produced by a nitrogen generator (Dalco Engineering, Dunshaughlin, County Meath, Ireland) was used to reduce the O₂ levels below ambient. The rest of the growth conditions were kept constant between the two treatments. All plants were grown under a 16 h/8 h simulated house conditions, 2–3 fully expanded leaves were tagged on each individual and the plants were then transferred into four BDW40 growth chambers that displayed no chamber effects (see Porter et al., 2015). Half of the plants were subjected to a replicated TJB atmospheric treatment in two of the chambers for 2 months, while the rest were grown under ambient atmospheric conditions for the same amount of time, serving as our controls (Supplementary Data Table S2). The atmospheric composition used for the TJB treatment was 16 % O₂ and 1900 ppm CO₂, which is a good approximation of the corresponding mean values for atmospheric O₂ and CO₂ across the boundary reported by mass balance modelling and palaeo-proxy studies (McElwain et al., 1999; Berner, 2001, 2006; Bergman et al., 2004; Belcher and McElwain, 2008; Bonis et al., 2010; Steinthorsdottir et al., 2011). The O₂ and CO₂ concentrations used for the ambient treatment were 21 % and 400 ppm, respectively (Table S1).

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Gas exchange measurements

Upon completion of the 2 month acclimation period, the responses of net assimilation rate to incident light (light curves) and intercellular CO₂ partial pressure (A–Cᵢ curves) were recorded within the chambers with a CIRAS-2 gas analyser (PP-Systems) attached to a PLC6(U) cuvette fitted with a 4.5 cm² measurement window and a red/white light LED unit. Theoretically, measuring within the TJB chambers at 16 % O₂ could introduce error in our measurements due to band-broadening effects; however, it has been shown that these effects are negligible even when using O₂-free air (Loriaux and Welles, 2004). Measurements were performed on intact leaves between 09:00 and 12:00 h to avoid potential mid-day stomatal closure. Air flow, leaf temperature and vapour pressure deficit during both the light and A–Cᵢ curves were maintained at 300 cm⁻¹ min⁻¹, 20 °C and 1·0 ± 0·2 kPa, respectively.

For the light response curves, tagged leaves from 3–4 plants per species and treatment were enclosed in the cuvette and illuminated at either 1200 (O. claytoniana, C. australis and C. oldamii) or 1600 (G. biloba and D. winteri) μmol m⁻² s⁻¹ until full photosynthetic induction, as judged from three consecutive stable readings of CO₂ assimilation (A) and stomatal conductance (gₑ), usually within 30 min. The CO₂ and O₂ concentrations used were identical to the corresponding growth values for each treatment (ambient, 21 % O₂–400 ppm CO₂; TJB, 16 % O₂–1900 ppm CO₂). Light levels were then adjusted from 1200/1600 to 20 μmol m⁻² s⁻¹ in nine/ten descending steps, each with a 3 min duration, which was always adequate to obtain stable A readings. The light-saturated photosynthetic rate (A_sat) and saturating light intensity were then calculated according to Norman et al. (1992).

For the A–Cᵢ curves, the tagged leaves previously used to acquire photosynthetic light response curves were again enclosed in the cuvette and allowed to equilibrate at 400 ppm CO₂, growth O₂ concentration (ambient, 21 % O₂; TJB, 16 % O₂) and saturating light intensity (calculated from the light response curves) for 30 min. CO₂ concentration in the cuvette (Cᵢ) was then stepwise decreased from 400 to 50 μmol mol⁻¹ (400, 300, 200, 150, 100 and 50) and then increased from 50 to 2000 μmol mol⁻¹ (50, 400, 500, 600, 800, 1000, 1200, 1600 and 2000). Relative stability of Cᵢ and A values at each step typically took 4 min, while a close agreement between the two measurements taken at 400 μmol mol⁻¹ indicated that exposure to low Cᵢ had not affected the activation state of Rubisco (von Caemmerer and Edmondson, 1986; Ethier and Livingston, 2004). The resultant response curves were fitted using the model equations of Long and Bernacchi (2003). Implementation of the model allowed the calculation of the maximum Rubisco-limited rate of carbon fixation (V_cmax) and the maximum electron flow rate supporting RuBP regeneration (J_max). Respiration in the light (R_d) was also calculated as the y-axis intercept of the A–Cᵢ response curve. We have to note here that the V_cmax and J_max values reported in our study are adjusted at 25 °C using the temperature functions of Bernacchi et al. (2001, 2003). A–Cᵢ curves obtained using dried leaves of each species were used to...
correct all measurements for CO2 leakages (Long and Bernacchi, 2003; Muir et al., 2014).

Incident growth light intensity (Q) for each species was measured with an MQ-200 quantum sensor (Apogee Instruments, Inc., Logan, UT, USA) and was then used to calculate the in situ electron transport rates from the non-rectangular hyperbola that describes the relationship between photon flux and electron transport (von Caemmerer, 2000) as:

\[ J_{\text{situ}} = \frac{Qz + J_{\text{max}} - \sqrt{(Qz + J_{\text{max}})^2 - 4QzJ_{\text{max}}}}{2Qz} \]  

(1)

where \( \theta \) is an empirical curvature factor with an average value of 0.7 (Evans, 1989), \( J_{\text{max}} \) is the maximum electron transport supporting RuBP regeneration and \( Qz \) is the light utilized by photosystem II (PSII) and is calculated as:

\[ Qz = Q \times \text{abs} \times \Phi_{\text{PSII max}} \times 0.5 \]  

(2)

(Long and Bernacchi, 2003) where \( Q \) is the incident photosynthetically active radiation, \( \text{abs} \) is the leaf absorptance and \( \Phi_{\text{PSII max}} \) is the maximum efficiency of primary photochemistry. A common absorptance value of 0.85 was used in our calculations (von Caemmerer, 2000), and \( \Phi_{\text{PSII max}} \), which is equivalent to \( F_s/F_m \), was measured with a continuous excitation fluorimeter (see below). Average values of \( Q, F_s/F_m \), and \( J_{\text{max}} \) were used for the calculation of \( J_{\text{situ}} \) at the species and treatment level.

In situ rates of ribulose 1,5-bisphosphate (RuBP) oxygenation (\( VO \)) and carboxylation (\( VC \)) were calculated from the corresponding light curve recordings (i.e. the step of the light curve with an intensity close to that received by each species in situ) according to Sharkey (1988) as:

\[ V_O = (A + R_d)/([1/\varphi] - 0.5) \]  

(3)

\[ V_C = A + 0.5 \times V_O + R_d \]  

(4)

\[ \varphi = 2 \times \Gamma^*/C_c \]  

(5)

Chloroplastic CO2 concentrations (\( C_c \)) were calculated from the corresponding \( C_1 \) values as:

\[ C_c = C_1 - A/g_m \]  

(6)

Mesophyll conductance (\( g_m \)) was calculated using the constant \( J \) modelling method of Harley et al. (1992). Five measurements of the RuBP regeneration-limited phase (typically at \( C_1 \) values between 50 and 120 Pa) of each A–C response curve were used to calculate the photosynthetic linear electron flow rate (\( J \)) as:

\[ J = (A + R_d) \times \frac{4 \times (C_i - A/g_m) + 2 \times \Gamma^*]}{(C_i - A/g_m) - \Gamma^*} \]  

(7)

Given that \( J \) is constant when \( A \) is limited by the regeneration rate of RuBP, the \( g_m \) value that minimizes the variance in \( J \) was calculated iteratively using the Solver Microsoft Excel add-in (Warren, 2006).

\( \Gamma^* \) is the photorespiratory compensation point (i.e. the chloroplastic CO2 concentration at which photosynthesis equals photorespiration) and depends on the temperature-sensitive relative affinity of Rubisco for CO2 and O2. \( \Gamma^* \) is considered to be relatively conserved among C3 species and is linearly correlated with O2 concentration; thus, we assigned standard values of 3-16/2-41 Pa at 20 °C and 21 %/16 % O2, respectively, for all five species in our study (Bernacchi et al., 2002).

Chlorophyll fluorescence measurements

Chlorophyll fluorescence measurements were performed on all three tagged leaves of each plant for a total of 12–18 measurements per species and treatment. After dark-adapting the leaves for 1 h, a Pocket-Pea continuous excitation fluorimeter (Hansatech Instruments Ltd, Kings’ Lynn, Norfolk, UK) was used to capture their fast chlorophyll \( a \) fluorescence transients. Saturating light (approx. 3500 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \)) was provided by a single high intensity red LED (peak at 627 nm), and chlorophyll fluorescence values were recorded from 10 \( \mu \text{s} \) to 1 s. Data acquisition rates were 105, 104, 103, 102 and 10 readings per second in the time intervals of 10–300 \( \mu \text{s} \), 0.3–3 ms, 3–30 ms, 30–300 ms and 0.3–1 s, respectively. The cardinal points of the recorded polyphasic fluorescence kinetics [OJIP curves, cardinal points: fluorescence value at 20 \( \mu \text{s} \) (\( F_o \)), fluorescence value at 300 \( \mu \text{s} \) (\( F_{300 \mu \text{s}} \)), fluorescence value at 2 ms (\( F_l \)), fluorescence value at 30 ms (\( F_l \)) and maximal fluorescence intensity (\( F_{m} \)) were then used to derive the following parameters according to the JIP-test (Strasser et al., 2004), as extended to include the effect of events related to the final electron acceptors of PSI (Tsimilli-Michael and Strasser, 2008):

\[ (1) \quad F_{l}/F_{m} = (F_{m} - F_{o})/F_{m} \quad \text{or maximum quantum yield of primary photochemistry, } \]

\[ F_{l}/F_{m} \] is a sensitive indicator of stress conditions with typical values of around 0.83 for healthy plants (Bjorkman and Demming, 1987; Johnson et al., 1993).

\[ (2) \quad DL_{J/R}C = (ABS/RC) - (TR_{J/R}C) \]  

is the heat dissipation per reaction centre at time zero.

where:

\[ \text{ABS/RC} = (M_o/V_i)/(1 - F_o/F_{m}) \]  

is the absorption energy flux per PSII reaction centre. \( TR_{J/R}C = M_o/V_i \) is the trapping per reaction centre at time zero.

and

\[ M_o = 4 \times (F_{300 \mu \text{s}} - F_o)/(F_m - F_o) \]  

is the slope at the origin of the fluorescence rise. \( V_i = (F_{2 ms}/F_o)/F_{m}/F_o \) is the relative variable fluorescence at 2 ms.

Statistical analysis

Statistical analysis was performed in R (v.3.1.1). Data were tested for normality and equal variance, and analysed using mixed effects models. Chamber identity was treated as a random effect to identify a possible chamber effect (no chamber effect was detected). Multiple models were run with random and interaction effects. Models were compared using analysis of variance (ANOVA) comparison, and the best fit model was determined using the Akaike information criterion (AIC).
One-way ANOVA and Tuckey post-hoc analysis were performed to assess the significance level of the differences between all fixed effects (i.e. species and treatments) from the best fit model.

RESULTS

Light response curves indicated that the $A_{\text{sat}}$ of the test species did not acclimate uniformly to low $[O_2]:[CO_2]$ (Fig. 1). Even though the photosynthetic stimulation was statistically significant only in *C. oldhamii*, the two ferns and the two angiosperms increased their $A_{\text{sat}}$ by 13-6-43-0 % depending on the species, while the gymnosperm *G. biloba* displayed a small, non-significant decrease (6-1 %). We should note here that *Ginkgo* exhibited a decrease in its light-saturated photosynthetic rate despite the fact that light curves for all species were taken at growth CO2 concentration, i.e. nearly 5-fold higher

non-significant decrease ($6 \pm 00$ % depending on the species). The error bars denote 1 s.d. Asterisks indicate statistically significant differences between treatments for each species ($P \leq 0.05$).

**Fig. 1.** Light-saturated photosynthesis ($A_{\text{sat}}$) of the test species under ambient (400 ppm CO2, 21 % O2) and TJB (1900 ppm CO2, 16 % O2) atmospheric conditions (leaf temperature = 20 °C). $n = 3$–4 depending on the species. The error bars denote 1 s.d. Asterisks indicate statistically significant differences between treatments for each species ($P \leq 0.05$).

One of the most important effects of low $[O_2]:[CO_2]$ was confirmed by the results of the $A_{\text{sat}}$ of the test species (Table 1). Acclimation to low $[O_2]:[CO_2]$ led to significant decreases in $V_{C_{\text{max}}}$ and to a lesser extent $J_{\text{max}}$ values in all test species; however, the decreases were most prominent in *G. biloba*, reaching 64.8 and 57.8 %, respectively, relative to the corresponding values of the control plants (Table 1). As a result of these changes, almost all species exhibited a decreased $V_{C_{\text{max}}}/J_{\text{max}}$ ratio under low $[O_2]:[CO_2]$ (Table 1), which is indicative of an altered balance between RuBP carboxylation and regeneration typically observed in plants growing at high CO2 (Long *et al.*, 2004; Ainsworth and Rogers, 2007; Osada *et al.*, 2010). Yet, we should note that only in three of the species was this decrease statistically significant.

In situ carboxylation rates ($V_{C}$) did not display significant changes, despite the decrease in $V_{C_{\text{max}}}$, due to the much higher growth CO2 concentration of the TJB treatment (Table 1). As expected, in situ oxygenation rates of Rubisco ($V_{O}$) diminished due to the very low $[O_2]:[CO_2]$ ratio of the growth air mixture in combination with the altered absolute concentrations of both gases (Table 1), yet overall *G. biloba* was the only species to display a significantly decreased combined rate of Rubisco carboxylation/oxygenation under TJB atmospheric conditions ($V_{C} + V_{O}$, Table 1). Furthermore, *G. biloba* was also the species that displayed the most significant reductions in absolute ($V_{O}$) and relative ($V_{O/V_{C}}$) rates of oxygenation primarily as a result of its extraordinarily high rates of Rubisco oxygenation under ambient conditions (Table 1). Indeed, calculation of the total conductance ($g_{l}$) from the measured values of $g_{s}$ and $g_{m}$ revealed that under ambient atmospheric CO2 *G. biloba* poses substantially higher resistances to CO2 diffusion and its photosynthetic machinery operates under significantly lower $C_{i}$ compared with the rest of the test species (Fig. 2A, B).

Ribulose-1,5-bisphosphate is the substrate for both carboxylation and oxygenation reactions of Rubisco, thus the reduction of the combined Rubisco carboxylation/oxygenation rate observed in the TJB treatment *Ginkgo* plants should normally be accompanied by a proportional reduction in the in situ rates of electron transport supporting RuBP regeneration ($J_{\text{situ}}$). Indeed, $J_{\text{situ}}$ values followed a similar pattern, showing small, non-significant changes between control and TJB plants in ferns and *C. oldhamii*, moderate decrease in *D. winteri*, a substantial decrease in *G. biloba* (Table 1) and correlated with the corresponding decreases in $V_{C} + V_{O}$ (Fig. 3A). It is interesting, however, that when the relatively small changes in $V_{C}$ values are ignored and the changes in $J_{\text{situ}}$ are plotted against the corresponding changes in $V_{O}$, the correlation becomes more robust (Fig. 3B). In addition, there also seems to be good correlation between the relative decreases in the in situ $V_{O}$ [Rel. $DV_{O} = (V_{O_{\text{amb}}} – V_{O_{\text{TJB}}})/V_{O_{\text{amb}}}$] and the decreases of Rubisco content-dependent $V_{C_{\text{max}}}$ when plants are exposed to the TJB atmospheric treatment (Fig. 4).

Exposure to the TJB treatment led to plant group-specific changes in $F_{r}/F_{m}$ (Fig. 5A). Compared with controls, the two fern species maintained high $F_{r}/F_{m}$ values while angiosperms showed moderate, yet non-significant decreases. *Ginkgo biloba* was the only species to display a substantial drop in its $F_{r}/F_{m}$, which indicated that low $[O_2]:[CO_2]$ acclimation resulted in partial photoinhibition. Interestingly, changes in $F_{r}/F_{m}$ were found to correlate linearly with changes in both $J_{\text{situ}}$ and $V_{O}$ (Fig. 6A, B). Furthermore, in the case of *G. biloba*, the substantial decrease in the maximum quantum yield of primary photochemistry was accompanied by a 2.5-fold increase in the rate of heat dissipation per PSII reaction centre (Fig. 5B). This clearly suggested a severely decreased efficiency of photosynthetic light reactions in the low $[O_2]:[CO_2]$-acclimated plants.

**DISCUSSION**

Our results clearly demonstrate that *Ginkgo biloba* diverts an extraordinarily high percentage of the ATP and NADPH produced during the photosynthetic light reactions to photosynthetic metabolism when grown under ambient CO2 and O2 concentrations (Table 1). This is contrary to the pattern observed in the two fern and two angiosperm species studied. Inherent (Yiotis *et al.*, 2010) as well as stress-induced (Flexas...
et al., 2006) diffusional limitations have been proven to result in a substantial drawdown in $C_s$ concentrations and high photorespiration rates. Given that the $O_2$ concentration in the chloroplast is considered to be about equal to ambient under any circumstance, low stomatal and/or mesophyll conductance to CO2 diffusion could lead to decreased chloroplastic CO2/O2 ratios and thus increased relative rates of $V_O$. Indeed, our results showed that G. biloba poses significantly higher resistance to CO2 diffusion compared with the basal angiosperms and ferns of the study (Fig. 2A, B). This seemingly peculiar and extremely low conductance to CO2 resembles the decreased conductance observed in plants under stress, but it can be explained by the limited ability of G. biloba to adapt to low [CO2] by altering its conductance to CO2 diffusion. This is supported by recent evidence that the dynamic range of stomatal conductance of Ginkgo is constrained due to its low anatomical $G_{max}$ (McElwain et al., 2016) and the fact that the mesophyll conductance of gymnosperms is generally low (Flexas et al., 2012).

Yet, $C_s$ does not depend solely on a species’ resistance to CO2 diffusion. $C_s$ is a function of both $g_t$ and $A$ (von Caemmerer, 2000); thus, in spite of the anatomical and/or
show that there is good correlation between the decreases in $V_O$ and the Rubisco content-dependent $V_{C_{max}}$ (Fig. 4). Moreover, due to the sheer amount of leaf nitrogen invested in Rubisco (Evans, 1989), we could argue that $V_{C_{max}}$ generally reflects plant nitrogen content and nitrogen assimilation capacity (Walker et al., 2014). Concomitantly, the correlation between the relative decreases in $V_O$ and $V_{C_{max}}$ seems to support the proposed link between photorespiration and nitrogen assimilation.

Plants have a complex control mechanism that adjusts the rate of the ‘light photosynthetic reactions’ and the combined rate of photosynthesis and photorespiration so that they match each other (Sharkey, 1990; Zhang and Portis, 1999; Andersson, 2008; Parry et al., 2008); however, stress conditions can slow down the rate of the Calvin-Benson-Bassham cycle, thus generating a potentially harmful imbalance. In these cases, photorespiration is believed to act as a safety valve, quenching the excess absorbed light energy by consuming the produced ATP and NADPH that cannot be used for carbon assimilation. Exposure to a TJB treatment, however, diminished photorespiration (Table 1; Fig. 7) and in conjunction with the stable or decreased in situ $V_C$ (Table 1; Fig. 7) led to a drop in the demand for RuBP regeneration, which is reflected in the corresponding drops in operational $J_{situ}$ (Table 1; Figs. 3A, B and 7).

Under these conditions of reduced capacity for photosynthetic and photorespiratory quenching of absorbed light energy, the electron transport chain of the light reactions becomes over-reduced and plants are forced to increase the efficiency of energy quenching through heat dissipation in order to avoid permanent photodamage (Demmg-Adams and Adams, 1996; Horton et al., 1996; Müller et al., 2001; Lambrev et al., 2012). The amplitude of this increase depends on the amount of excess absorbed energy or, in our case, the drop in the capacity for photosynthetic and photorespiratory quenching, assuming no significant changes in leaf absorption. Indeed, under low [O$_2$]:[CO$_2$], $G. biloba$ displayed the highest increase in the heat dissipation per reaction centre ($D_{I_o/RC}$) compared with the rest.
of the species, with ferns and angiosperms only showing negligible changes (Figs 5B and 7). Even so, it appears that the increased efficiency of heat dissipation is not adequate to alleviate photoinhibition, as revealed by the significant decrease of $F_v/F_m$ in *G. biloba* under TJB atmospheric conditions (Figs 5A, 6A, B and 7).

Our results do not allow us to identify the increased rate of photorespiration in *G. biloba* either as proof of the species’ reduced fitness to current low atmospheric CO₂ or as an evolutionary strategy aiming to enhance the species’ persistence to stress and/or nitrogen assimilation capacity. Yet, it is apparent that exposure to a low [O₂]:[CO₂] atmosphere and subsequent diminishement of the photorespiratory sink for photosynthetic electron flow severely affects the species’ competitiveness, especially when compared with the fern species, which display a high level of photosynthetic plasticity. In contrast to *G. biloba*, ferns and angiosperms displayed a remarkable adaptability of their physiology to TJB atmospheric conditions. Ferns’ adaptive decreases in $V_{\text{Cmax}}$ and $J_{\text{max}}$ (Table 1) were counterbalanced by the increased CO₂ concentration in such a way that both the *in situ* $V_C$ and $A_{\text{sat}}$ showed an increasing, yet non-statistically significant, trend (Table 1; Fig. 1). In addition, the absence of changes in the heat dissipation per reaction centre ($DI_v/RC$) and the maximum efficiency of primary photochemistry ($F_v/F_m$) under nearly non-photorespiratory conditions further highlights their ability to acclimate their physiology effectively to low [O₂]:[CO₂] (Figs 5A, B and 7).

We acknowledge that an increase in global temperature and high atmospheric CO₂-induced decreased transpiration of the broadleaved Ginkgoales must have played a role in their decline across the TJB (McElwain et al., 1999). However, the present work provides an additional mechanism that may have contributed to the near extinction of Ginkgoales and to the proliferation of ferns evident in the TJB fossil record (Fowell and Olsen, 1993; Olsen et al., 2002; McElwain et al., 2007). Our study focused exclusively on innate differences in the photosynthetic plasticity and did not investigate the anatomical
adaptations of leaves grown under a TJB atmosphere. Yet, we would like to note that after >100 million years of plant evolution and despite the plummeting of atmospheric CO2 levels, gymnosperms still display relatively low values of stomatal and mesophyll conductance, even though higher values would have enabled them to maintain higher photosynthetic rates and/or higher photosynthetic nitrogen use efficiency (Flexas et al., 2012; McElwain et al., 2016). Based on this apparent reduced anatomical/physiological adaptability of gymnosperms, we believe that the responses of present-day G. biloba to a TJB treatment are likely to resemble those of Ginkgoales present during the TJB. Overall, our study stresses the importance of differences in the physiological plasticity of different plant groups in shaping evolutionary patterns under fluctuating atmospheric O2 and CO2. Thus, in light of ongoing rapid increases in atmospheric CO2 levels, we argue that further investigation of the innate physiological characteristics and constraints of different plant groups are of considerable significance as we are likely to be on the verge of major shifts in the composition of plant communities.

**SUPPLEMENTARY DATA**

Supplementary data are available online at https://academic.oup.com/aob and consist of the following. Table S1: classification, size of pots, compost mix and fertilizer used for each of the studies. Table S2: mean values ± s.d. of growth conditions in the chambers used in the study.

**ACKNOWLEDGEMENTS**

We thank Miss Bredagh Moran and Mr Gordon Kavanagh for their technical assistance. This work was supported by the European Research Council [grant no. ERC-2011-StG 279962-OXYEVOL].

**LITERATURE CITED**


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